THE MYCORRHIZAL PLANT ROOT SYSTEM: FORAGING ACTIVITIES AND INTERACTION WITH SOIL BACTERIA IN HETEROGENEOUS SOIL ENVIRONMENTS

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ZUSAMMENFASSUNG

Der Beitrag der arbuskulären Mykorrhizapilze zur Nährstoffaufnahme und zum Wachstum von Pflanzen ist vom Genotyp des Pilzes und der Pflanze abhängig, sowie von den Umweltbedingungen. In der vorliegenden Arbeit wurden Mykorrhizapilze unterschiedlicher Herkunft verwendet. Im Mittelpunkt der Arbeit stand die Untersuchung der Rolle der Mykorrhiza bei der Reaktion der Pflanze auf räumlich unterschiedliches Nährstoffangebot im Boden. Als Versuchspflanzen wurden Süßkartoffel und Tagetes verwendet.

Für die Arbeit wurden verschiedene Modellexperimente durchgeführt. In speziell für diese Arbeit konstruierten Gefäßen wurden nicht-mykorrhizierte und mykorrhizierte Süßkartoffelpflanzen mit organischer Substanz versorgt, die entweder gleichmäßig oder heterogen im Substrat verteilt war. In weiteren Experimenten wurde mit Hilfe von "split-root" Systemen die Wirkung arbuskulärer Mykorrhizapilze auf ein lokales Angebot von mineralischem Phosphor und Stickstoff im Boden untersucht. Darüber hinaus wurde in Versuchen Kompost räumlich konzentriert im Substrat angeboten. Die Messungen umfassten den Mykorrhizierungsgrad der Wurzel, die Entwicklung des extraradikalen Myzels, die Trockenmasse der Pflanze sowie die Konzentrationen an Phosphor und Stickstoff in der Pflanze.

Eine Besiedlung der Wurzeln mit arbuskulären Mykorrhizapilzen führte in den meisten Versuchsansätzenzu einer erhöhten Nährstoffaufnahme der Pflanze und zu einem verbesserten Wachstum. Ein besonders starkes Hyphenwachstum in Bodenzonen mit viel organischer Substanz wurde jedoch nicht beobachtet. Zugabe von Kompost führte teilweise zu einem Rückgang des Mykorrhizierungsgrades.

Die Verwendung von organischem Material oder Kompost im Gartenbau kann sinnvoll sein und zur Verminderung von Mineraldüngung beitragen. Optimales Pflanzenwachstum und Mykorrhizawirkung erfordern jedoch eine gute Balance zwischen Art und Menge des organischen Stoffes bzw. Komposts, den Substrateigenschaften und den Pflanzen- und Pilzgenotypen.

SCHLAGWÖRTER

Arbuskuläre Mykorrhizapilze, Heterogene Nährstoffverteilung im Boden, Kompost, Organische Substanz, Phosphor, Süßkartoffel, Stickstoff, Tagetes

ABSTRACT

The actual contribution of arbuscular mycorrhizal (AM) fungi to plant nutrient uptake depends on the fungal and plant genomes, and on environmental conditions. In the present study, AM fungi of different origin, for example isolated from plots with different long-term fertilizer application history, were used to quantify their contribution to plant nutrient uptake under situations of spatially heterogeneous soil nutrient distribution. Test plants for this study were sweet potato and marigold.

Several model experiments were carried out. In specifically constructed growth containers, non-mycorrhizal and mycorrhizal sweet potato plants were supplied with organic matter either homogeneously or heterogeneously distributed in the substrate. Bacteria from a long-term organically fertilized soil were also added as a treatment. In other experiments using a split-root approach, the influence of AM fungi on the plant response to localized mineral phosphorus and nitrogen supply was studied. In a further experiment, the effects of localized compost supply on marigold plants inoculated with *Glomus mosseae* were investigated.

Arbuscular mycorrhizal fungi increased nutrient uptake and growth of plants under most conditions, also when nutrients were heterogeneously distributed in soil. However, there was no indication of increased hyphal proliferation or activity in soil spots with high organic matter. Plant phosphorus status regulated the extent of AM root colonization. The extent of AM root colonization was partly decreased by application of organic matter and of compost to the substrate.

Application of organic matter and/or compost can be beneficial in horticulture and can replace mineral fertilizer use. However, optimum plant growth and mycorrhizal function require a good balance between type and amount of organic matter or compost, growth substrate properties and plant and AM fungal genotype.

KEYWORDS

Arbuscular mycorrhizal fungi, compost, heterogeneous soil nutrient distribution, marigold, nitrogen, organic matter, phosphorus, sweet potato

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ABBREVIATIONS

AM	arbuscular mycorrhiza(l)
+B	with bacteria inoculation
-B	without bacteria inoculation
CEC	cation exchange capacity
DW	dry weight
DS	dry soil
GM	*Glomus mosseae*
GI	*Glomus intraradices*
HC	hyphae compartment(s)
HP	high level of phosphorus
Hm	homogeneously distributed
Ht	heterogeneously distributed
IP	inside patch
Le	leaf material
LeHm	leaf material homogeneously distributed
LeHt	leaf material heterogeneously distributed
LP	low level of phosphorus
+M	mycorrhizal treatment
-M	non-mycorrhizal treatment
MHB	mycorrhiza helper bacteria
MM	AM fungi from minerally fertilized soil
MO	AM fungi from organically fertilized soil
NM	non-mycorrhizal
OP	outside patch
PGPR	plant growth promoting rhizobacteria
PSB	phosphorus solubilising bacteria
RC	root compartment(s)
SD	standard deviation
St	stem material
StHm	stem material homogeneously distributed
StHt	stem material heterogeneously distributed

1. GENERAL INTRODUCTION

In developing countries, the economy is still based on the agricultural sector. For small and marginal farmers, the use of chemical fertilizers is often costly. In addition, the excess use of chemical fertilizers has contributed to pollution and contamination of soils and water, can harm microorganisms in soil and may reduce long term soil fertility. Application of organic matter, such as livestock manure, green manure or compost, and of biofertilizers (microorganisms beneficial for plant nutrient uptake) may be an alternative to the use of chemical fertilizer. Using organic matter instead of chemical fertilizers can also contribute to the reduction of non-renewable resources use in the chemical fertilizer production processes.

One group of microorganisms often recommended as biofertilizers are the arbuscular mycorrhizal (AM) fungi which occur commonly in the roots of most plant species. Hyphae of AM fungi enhance the uptake of phosphorus and other nutrients that are required in large amounts for plant growth. The effectiveness of AM fungi to contribute to plant nutrient uptake is often found to vary, depending on fungal genome and soil conditions. Several soil properties are important factors for the colonization, growth and distribution of AM fungi that directly or indirectly influence plant nutrient uptake.

In the frame of the present thesis, some soil conditions that influence AM fungi in their contribution to plant nutrient uptake and hence plant growth were studied. A short general introduction into the topics of this work is given in this section. Each experimental chapter of the thesis presents an individual introduction to the specific topic of the respective chapters.

1.1 THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

Arbuscular mycorrhiza is a mutualistic symbiosis between soil fungi from member of Glomeromycota and roots of the large majority of vascular terrestrial plants (Genre et al., 2005). The classification of arbuscular mycorrhizal fungi is under discussion at present. This thesis uses the conventional classification of the past decades.

1.1.1 BIOLOGY AND ECOLOGY OF ARBUSCULAR MYCORRHIZAL FUNGI

Among the several mycorrhizal associations, the arbuscular mycorrhiza is characterized by highly branched fungal structures, the arbuscules, which grow intracellulary

without penetrating the host plasmalemma (Pichardo et al., 2012). Approximately 80% of vascular plant species, including most angiosperms and gymnosperms (Genre et al., 2005), are capable of forming an AM symbiosis (Smith and Read, 2008, p.3). In this symbiosis, the fungi receive their carbon as energy source from their host plant. At the same time, the host plants receive part of their nutrients from the soil via hyphae of the fungi. By an extensive hyphal network outside the nutrient depletion zone around the root, a larger soil volume can be exploited by AM plants compared to non-mycorrhizal plants (Richardson et al., 2011). Plant uptake of nutrients such as N, P, K, Ca, Mg, Zn, Cu, and Mn can be elevated after forming an AM symbiosis (Tong et al., 2006). The AM symbiosis can also enhance the plant tolerance against some unfavorable environmental conditions (Medina and Azcón, 2010).

The AM fungi are unable to complete their life cycle without the establishment of the symbiosis (Smith and Read, 2008, p.17). Based on the degree of benefits received from the mycorrhizal association, plant species can be categorized as obligatory, facultative and non-mycorrhizal (Brundrett, 2002). Plants that rely on the AM symbiosis for nutrient uptake typically have coarse, fibrous root systems with few root hairs. In contrast, plants that have finer root systems with abundant root hairs can often absorb nutrients independent of AM fungi (Miller and Kling, 2000).

There are three important components of the mycorrhizal root system: the root itself, the fungal structure within the root (arbuscules, coils, vesicles, intraradical mycelium) and the extraradical mycelium (the fungal structure within the soil). The extraradical mycelium explores and exploits the soil for nutrients and then transports those nutrients to the root (Kuyper et al., 2004). In the mycorrhizal root, the exchange between nutrients from the fungus and carbon from the plant occurs in arbuscules (Bever et al., 2001). Vesicles contain lipids and cytoplasm and act as carbon storage compartments for the fungi. However, not all members of the Glomeromycota form vesicles in their association. Therefore, the term "arbuscular mycorrhizal (AM) fungi" is now preferred by many researchers to represent this association rather than the previously used term "vesicular-arbuscular mycorrhizal (VAM) fungi" (Habte and Osorio, 2004).

The plant root can be colonized by AM fungi from different sources of inoculum: spores, colonized root fragments and hyphae (Schalamuk and Cabello, 2010). The roots of host plant species release signalling molecules, known as strigolactones, that stimulate hyphal branching in AM fungi (Akiyama et al., 2005).After stimulation, hyphae make contact with roots and this is followed by adhesion and formation of appressoria. Thereafter, infection hyphae develop from appressoria and penetrate the outer root tissue (Genre et al., 2005).

After initial infection, AM fungi form additional infection units to extend the fungal colony within the root system. This enables the fungi to obtain carbon from their host plant, and continue the development of extraradical mycelium (Sbrana, 2006).

The degree by which mycorrhizal fungi can enhance plant nutrient uptake depends on biotic and abiotic factors that influence the plant host, the fungi and their association (Habte and Osorio, 2004). Species or isolates of AM fungi associated with a particular plant have different abilities to promote plant growth and nutrient uptake (Smith et al., 2004). The differences between AM fungi in their contribution to growth of an associated plant may be related to differences in their capacity to develop an extraradical hyphal system (Garcia-Garrido et al., 2000), although greater hyphal density is not of equal significance for uptake of all ions from soil (George, 2000).

The situation is even more complex though. When different plants species are colonized by the same AM fungus, this usually does not result in similar plant growth responses. Plant growth response depends, among other factors, on the size of the benefit to colonized plants (P supply to plants) and the size of the costs of the AM fungus (C supply to fungus) (Smith et al., 2011).

Abiotic factors such as P concentrations in soil also affect the mycorrhizal symbiosis. High P concentrations in soil inhibit AM fungal root colonization of host plants and the growth of extraradical hyphae in soil.The adverse effect of high P concentrations in soil on AM formation is correlated with a reduction in the delivery of soluble carbohydrates to AM fungi (Olsson et al., 2006). In addition, at high soil P supply roots grow faster than the rate at which they can be colonized by AM fungi (Richardson et al., 2011). High N concentration in soil can also decrease AM fungal root colonization (Blanke et al., 2005). However, Vázquez et al. (2001) reported that high N concentration in soil did not affect the AM fungal root colonization. Furthermore, Garcia et al. (2008) even reported that N fertilization was associated with a significant increase in AM colonization. They suggested that N fertilization increases AM root colonization when the phosphorus status of the plant host is low.

1.1.2 FORAGING ACTIVITIES OF ARBUSCULAR MYCORRHIZAL ROOTS IN A HETEROGENEOUS SOIL ENVIRONMENT

The root system is fundamentally important for plant growth and survival because its role in water and nutrient uptake (Osmont et al., 2007). Plant nutrient uptake is strongly dependent on the total absorptive surface area of the root system (Eissenstat and Volder, 2005). The development of the root system of plants is controlled by the plant genome but it can be modified by factors of the environment where roots grow (McMichael et al., 2011).

Ecological science assumes that well adapted root systems have the ability to maximize the acquisition of resources from their environment. In natural soil, spots with high nutrient availability are heterogeneously distributed in soil (Lima et al., 2010). Plants often respond to heterogeneous nutrient distribution in soil by producing significantly more roots within the nutrient rich zone/patch (root proliferation; Mommer et al., 2012). The amount and the speed of the response can vary among species (Weerasinghe and Tanner, 2006).

Root proliferation in nutrient rich patches can be interpreted in terms of a foraging response (Robinson, 2001). Not only macronutrients such as N, P, and K (Lambers et al., 2008, p.280) but also micronutrients such as Zn are able to stimulate root proliferation in patches (Whiting et al., 2000). By this response, plants become able to optimize the uptake of nutrients within this patch. Some studies have shown that plants grow better when nutrients are heterogeneously distributed in the soil compared to a situation where the same quantities of nutrients are homogeneously distributed in the soil (Kume et al., 2006; Roiloa and Retuerto, 2006).

The extent of root proliferation to exploit nutrient rich soil patches is controlled by the nutrient status of the plant (Desnos et al., 2008). Root proliferation in the nutrient rich zone is higher when the nutrient status of the plant is lower. However, in contrast, Bilbrough and Caldwell (1995) reported that plants with high nutrient status showed greater root proliferation in the nutrient rich patch than plants with lower nutrient status. They suggested that plants with higher nutrient status are more vigorous and thus exhibit a greater root growth response than plants with lower nutrient status. The response of plants to nutrient rich patches is also affected by other factors such as the size of the patch, the nutrient concentration in the patch, the type of nutrient, and the overall soil fertility (Wang and Cheng, 2004).

Besides root growth, plants also have ability to increase nutrient uptake capacity per unit root length when they encounter nutrient rich patches (Weerasinghe and Tanner, 2006).

Most roots of terrestrial plants are colonized by AM fungi, and as mentioned above nutrients in natural soil are heterogeneously distributed. It is therefore very necessary to note that plant response to heterogeneous nutrient distribution in soil may be modified by the symbiosis with AM fungi. The hyphae of AM fungi can extend the potential foraging zone of roots where root direct access is limited (Wijesinghe et al., 2001). Thus, AM fungi may assist their host plant in the exploitation of heterogeneously nutrient distribution, either by exploiting nutrient rich patches or by increasing nutrient uptake capacity outside the patch (Neumann and George, 2010).

Many researchers have shown that hyphae of AM fungi can proliferate in both organic (Hodge and Fitter, 2010) and inorganic (Cui and Caldwell, 1996; Olsson et al., 2006) nutrient rich patches. The proliferation of mycorrhizal hyphae within nutrient rich patches is more profitable than root proliferation in terms of carbon cost (Wang and Cheng, 2004). Consequently, the rate of mycorrhizal root proliferation in nutrient patches may be slower because the acquisition of nutrients from the patch is already supported by a network of mycorrhizal hyphae (Tibbett, 2000). However, Cui and Caldwell (1996) reported that the ability of AM hyphae both to acquire P from enriched soil patches and to deliver it to the host roots is similar in quantity to that in a situation with uniform nutrient distribution in soil. The hyphae of AM fungi may not continue to proliferate in the P rich patch unless the plant allocates carbon specifically to AM fungi in this patch (Olsson et al., 2006).

1.1.3 INTERACTION BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI AND BACTERIA TO INCREASE PLANT GROWTH

The AM symbiosis affects the community and diversity of the organisms present in the soil. By increasing the absorptive surface area of their host plant root system, the hyphae of these symbiotic fungi provide an increased area also for interaction with other microorganisms (Albertsen et al., 2006). The areas where that interaction can occur are the areas surrounding the roots and fungal hyphae, commonly referred to as the mycorrhizosphere (Artursson et al., 2006). The composition of the bacterial population in the mycorrhizosphere may be affected by exudates from plant roots and from extraradical mycelium of AM fungi. The differences in amount and composition of exudates from plant roots and from extraradical mycelium in fact play an important role in the selection of bacteria in the AM fungal plant association (Bharadwaj et al., 2008). Some results indicate that bacterial community structure in the mycorrhizosphere depends more on the AM fungi present than on host plant identity (Bonfante and Anca, 2009; Roesti et al., 2005). The

bacterial community can also be affected more indirectly, by root morphology, soil pH, soil nutrient content, soil enzyme activity, and soil structure (Marschner and Timonen, 2006).

In the mycorrhizosphere, plant beneficial bacteria may interact directly or indirectly with AM fungi to promote plant growth. These beneficial bacteria have been identified as (a) Mycorrhizal Helper Bacteria (MHB) (b) Phosphorus Solubilising Bacteria (PSB) and (c) Plant Growth Promoting Rhizobacteria (PGPR). The MHB promote the formation of the mycorrhizal symbiosis by stimulating extension of mycelia, increasing root-fungus contact, and by enhancing spore germination. The PGPR promote plant growth through direct and indirect interaction with the plant roots. The PGPR can improve plant growth by one or more mechanisms: direct stimulation of plant growth, enhancement of nutrient uptake, suppression of plant pathogens, and/or an induction of resistance in plant hosts against pathogens. The PSB mobilize phosphate ions from organic and inorganic P sources (Dames and Ridsdale, 2012).

Dual inoculation between of PGPR and AM fungi (Mäder et al., 2011) and of PSB and AM fungi (Prasad et al., 2012) increased the yield of inoculated plants further compared with plants inoculated either by AM fungi or beneficial bacteria alone. However, a screening to select the best microbe-host plant combination must be done in order to optimize results, because interactions between AM fungi and associated bacteria are highly specific (Jaizme-Vega et al., 2006). Jäderlund et al. (2008), for example, reported that different AM fungi react differently with the same bacterium when inoculated together. In addition, the concentration of the respective bacteria must be considered. A high concentration of bacteria seems to be harmful if not lethal to the AM fungus at least in some cases (Bonfante and Anca, 2009).

1.1.4 INTERACTION BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI AND ORGANIC MATTER

Fungi are heterotrophic. They do not have the ability to do photosynthesis and therefore the needs of their nutrition depend on the other organisms. Fungi can obtain their nutrients from dead or living organic substance, through decomposing of dead organic material (saprophytes), colonizing other living organisms causing disease or death (parasitism), or involvement in a mutualistic association (Brundrett, 1991).

In the AM fungal symbiosis, the fungi obtain soluble carbon from their host plant whereas plant nutrient uptake, particularly of P, is increased by an extended hyphal network in soil (Sunil et al., 2012). The AM fungi are able to exploit nutrients released from organic

matter during the decomposition process induced by other microorganisms (Alguacil et al., 2009), but were assumed not to be able to exploit P directly from organic matter (Joner and Jakobsen, 1995b).However, Hodge et al. (2001) reported that AM fungi increased N capture from dead organic material.

Thus, the ability of AM fungi to exploit nutrients directly from organic matter is still under debate (Dai et al., 2011).Thesaprotrophic capability of AM fungi is in any case limited because these fungi must obtain their energy directly from their host (Hodge and Fitter, 2010). It is also clear that AM fungi are unable to decompose dead organic matter. In contrast, ectomycorrhizal fungi and ericoid mycorrhizal fungi are able to decompose organic matter (Treseder and Cross, 2006).

The responses of AM fungi to organic matter amendment in soil depend at least partly on the quality and quantity of that organic matter (Linderman and Davis, 2001). The growth of AM fungi can be increased or decreased by organic amendment in soil. Their growth can be influenced directly by compounds released during the decomposition process or by secondary metabolites from microorganisms involved in organic matter decomposition (Gryndler et al., 2009). In a recent study, the proliferation of extraradical mycelium of AM fungi in soil was more increased by amendment of organic matter with narrow C:N ratio than by amendment of organic matter with wider C:N ratio (Dai et al., 2011).

In another study, root colonization and growth of extraradical mycelium of AM fungi were increased by application of sufficiently decomposed cellulose, but mycorrhizal symbiosis was inhibited by application of fresh cellulose or cellulose after shorter periods of decomposition (Gryndler et al., 2009). Cellulose is the main component in plant cell walls (Endler and Persson, 2011). Vaidya et al. (2007) reported that spore production of AM fungi was lower in a mesh bag with compost which contained high levels of P compared to a mesh bag with dried leaves from an agroforestry plant. Linderman and Davis (2001) reported that application or organic matter with high humic content to soil stimulated the mycorrhizal symbiosis.

In addition, organic amendment to soil can also indirectly influence AM fungal growth via influencing soil nutrient profile, soil structure, water holding capacity, and pH (Dai et al., 2011). The status of the organic matter content of the soil is important for mycorrhizal activity in general terms because the P availability in the soil has an important effect on mycorrhizal root colonization and spore production (Lakshmipathy et al., 2012). The AM fungal colonization is often suppressed by high concentrations of inorganic P, but not of organically-bound P (Linderman and Davis, 2001). Addition of organic matter to soil

decreases the bulk density of that soil and increases water holding capacity (Daynes et al., 2010). A decrease of soil bulk density usually causes increasing soil porosity, and mechanical resistance to hyphal growth may be reduced (Vaidya et al., 2008). Complex interactions then include the relationship of AM fungi, soil moisture, and plant root function (de Oliveira and de Oliveira, 2005*).* Root growth is inhibited in dry soil (DaCosta et al., 2004). An increasing water content in soil gives benefit to the mycorrhizal symbiosis as long as it is not causing a significant reduction in soil aeration (de Oliveira and de Oliveira, 2005)

1.2 EXPERIMENTAL PLANT SPECIES

1.2.1 SWEET POTATO

Sweet potato *(Ipomea babatas* (L.) Lamb.is a member of the Convolvulaceae family. The plant is generally characterized by starchy, succulent and tuberous storage roots, alternating palmately lobed leaves and medium sized sympetalous flowers which grow individually and vary in colour from white to varying degrees of purple. Its growth habit is predominantly prostrate with a vine system that rapidly expands horizontally on the ground (Titus et al., 2010, p.4). The plant can be propagated by using either generative or vegetative parts of the plant. However, vegetative propagation using either storage roots or stem cuttings is common (Huaman, 1999). Propagation using seeds is more difficult because it is difficult to produce seeds by self-pollination (Lebot, 2009, p.107).

Sweet potato has a wide range of adaptation to agro-ecological conditions and fits well into low-input agriculture (Egbe et al., 2012). However, the growth and yield of the storage root can be adversely affected by several environmental factors, including soil temperature, humidity, light, photoperiod, drought (Noh et al., 2013), and soil N availability (Villagarcia and Collins, 1998). Sweet potato is widely grown in tropical, subtropical and warm temperate regions (Srisuwan et al., 2006) and is grown mainly for its edible storage roots, although other parts of this plant can be consumed as a green vegetable, particularly the leaves and tips (Mortley et al., 2009). In developing countries, sweet potato is the fifth most important food crop after rice, wheat, maize and cassava (Veasey et al., 2008) because of high carbohydrate content in its storage root (Mortley et al., 2009). In addition, sweet potato is also used for animal feed (Lam and Ledin, 2004) and the starch of the storage root can also be used for industrial purposes (Mukherjee, 2002).

1.2.2 MARIGOLD

Tagetes patula L., also known as tagetes or French marigold, is an ornamental plant species belonging to the Asteraceae (or Compositae) family. It is native to South America but introduced and naturalized in most parts of the word. The characteristic of this plant is an annual growth habit with capitula flowers and alternate leaves, a height of stem of 30-60 cm with an upright and straight stem. It can grow in full sun and is sensitive to frost. It is flowering commonly in spring, summer and early autumn (Hassanpouraghdam et al., 2011). The present study used the cultivar "Mr Majestic" which is characterized by a red and yellow stripe in its petal. Marigold is commonly propagated from seed or as transplants (Tripepi et al., 2011).

Secondary metabolites of French marigold, particularly essential oils from both above-ground parts and roots, have been used as antibacterial, antifungal, insecticidal, nematicidal, and larvacidal agent (Hassanpouraghdam et al., 2011). In addition, this plant is also used as a cut flower or in borders of landscape settings (Valdez-Aguilar et al., 2009).

1.3 COMPOST

Compost is usually the product of controlled aerobic conversion of organic matter, resulting in stable, dark, brown, soil-like material (Rouse et al., 2008, p.17). However, compost can also be produced by anaerobic processes, although the rate of organic matter degradation is then lower and less efficient (Kuo et al., 2004). Anand et al. (2012) reported that the concentration of macronutrients such as N, P and K and of micronutrients in anaerobic compost was less than in aerobic compost. In general, the aerobic composting process is the preferred method to produce stable and mature compost (Kuo et al., 2004) and most useful for agricultural production (Naikwade et al., 2011).

Many organic materials are suitable to be composted. The ratio of carbon to nitrogen (C:N) of the organic material must be considered before composting because both C and N are needed by microbes in the composting process. The optimum C:N ratio of organic material in the composting process is in a range of 25:1 to 30:1, but composting has also been done in the C:N range of 20:1 to 40:1 (Seyedbagheri, 2010). The degradation of organic matter is not fast when the initial C:N ratio is over 40:1, while low C:N ratios tend result in an accumulation of NH_4-N as $(NH_4)_2CO_3$, promoting the volatilization of odorous NH_3 when the pH and temperature are elevated (Kuo et al., 2004).

The need for mineral fertilizer to improve plant growth and development can be reduced by an application of compost to soil. Thus, the environmental impact of fertilizer production, such as greenhouse gas emission, and the impact of phosphate extraction can be avoided (Prasad and Foster, 2009). On the other hand, large quantities of organic material that are treated as waste and have the potential to contaminate water resources, can be re-valued by composting (Seyedbagheri, 2010).

The amount of plant available nutrients released by compost is usually quite low. Mineral fertilizer may be required to support optimum growth and quality of commercial crops. Nevertheless, organic matter from applied compost improves the quality and fertility of soil, by improving of water retention, cation exchange capacity, soil structure and soil organic matter quality (Rivero et al., 2004). The benefit of compost application to plant growth and development depends on the maturity of compost. Mature compost is characterized by a pH between 7 and 9, a C/N ratio lower than 12 in the solid phase, an N-NH_4/ N-NO_3 ratio less or equal to 0.11% and a value of cation exchange capacity higher than 60 meq per 100 g of compost (Aina et al., 2012). Immature compost can easily be detected by its temperature and smell. Brinton (2001) summarised that immature compost has high temperature, smells poorly or does both. Also, immature compost still contains phytotoxic compounds such as NH_3 or short-chain organic acids (Gómez et al., 2006) which are deleterious to plant growth.

1.4 COMPOST TEA

Compost tea is a liquid extract from composted material that contains soluble plant nutrients in organic and inorganic form, and a large number of organisms including bacteria, fungi, protozoa and nematodes (Campbell, 2007, p.6). The use of compost tea has received some interest during the last decade in agricultural and horticultural practice (Al-Mughrabi, 2007). Application of compost tea may be a potential alternative to the application of mineral fertilizer, and of pesticides and fungicides (Dearborn, 2011). Thus, the use of synthetic products which may harm soil productivity, the ecosystem, and the groundwater can be eliminated (Hargreaves et al., 2008).

Based on the method used to produce the compost tea, there are two types of compost tea, aerated and non-aerated compost tea (Campbell, 2007, p.6). The production of compost teas is started by mixing solid compost with water with a ratio of solid compost to water in the range of 1:30 to 1:200. For aerated compost tea, the mixture of compost and water is

aerated by different means, while for non-aerated compost tea the mixture is not aerated. In both methods, sometimes supplemental nutrient sources for microbes are used such as molasses, algal powder, or yeast extract. The addition of microbial food during the production of compost tea is expected to increase microbial activity and effect (Arancon et al., 2007). The mixture is filtered to obtain an extract and then drenched into soil or sprayed onto foliage (Al-Mughrabi, 2007). Aerated compost tea can be produced in two to three days, while non-aerated compost tea may take up to two weeks to obtain good quality (Campbell, 2007, p.7).

Aerated compost tea is more commonly used as fertilizer and/or for nutrient mobilization than non-aerated compost tea because aerated compost tea can be prepared in a short in time and results in less odour problems. However, a quality difference between aerated and non-aerated compost tea in their effect on plant growth, yield and disease suppression cannot be generalized (Pant et al., 2011). Compared with compost application, compost tea use may be preferable for two reasons: to inoculate microbial life into soil or onto the foliage of plants, and to add soluble nutrients to the soil or to the foliage to directly feed the plants and the other organisms present (Ingham, 2005). Another reason of choosing compost tea over compost is that compost acts more slowly (Dearborn, 2011). Of course, the biochemical properties of compost tea are determined by the biochemical properties of the compost used as extracted material (Pant et al., 2011). In other words, the efficacy of compost tea to promote plant growth depends on the quality of compost used to make the compost tea. Compost with high microbial diversity has the potential to make a good compost tea (Campbell, 2007, p.13).

Soil quality and health are indicated by chemical and biological soil properties (Pant et al., 2011). Application of compost tea to the soil is designed to re-establish a healthy soil food web in degraded and toxic soils (Ingham, 2005). Compost tea is commonly applied to the soil by drenching it into the root zone (Campbell, 2007, p. 21). By application of compost tea to the soil, the numbers of active microbial population and the amount of mineral nutrients in the soil are increased. The active microbial population may play an important role in the subsequent soil organic matter mineralization (Pant et al., 2011). Moreover, beneficial microorganisms from the compost tea can compete for space and nutrients in the soil with harmful microorganisms that cause plant disease, can parasitize harmful microorganisms, and produce antimicrobial compounds, so that the development of plant root diseases can be suppressed (Koné et al., 2010).

Foliar application of compost tea is more effective to increase plant growth than soil application when under dry conditions.The soil has a lack of available water in the top. Compost tea contains often high amounts of nutrients (Zaller, 2006). Foliar application can be used for immediate impact in nutrient deficiency (Campbell, 2007, p.22) and to prevent foliar diseases (Ingham, 2005). However, compost tea use for foliar application requires fine filtration to prevent the clogging of sprayer nozzles, while compost tea for soil application does not require such filtration. Furthermore, foliar application has less effect on total population and diversity of microorganisms in the plant production system than soil application (Campbell, 2007, p. 21).

1.5 AIMS OF THE RESEARCH IN THE PRESENT THESIS

The overall aim of the work of this thesis was to describe the interaction of roots with soil microorganisms, in particular with AM fungi and bacteria, in their effect on plant nutrient uptake and plant growth promotion.

There have been very many previous studies on the effect of mycorrhizal colonization on plant nutrient uptake and growth. Similarly, bacterial inoculation effects on plant growth have also often been studied. The effects of heterogeneous nutrient supply have received much attention in ecological science in the last decades. The use of composts is not so much a research focus at present, but is advocated for in practical agriculture, in particular for biological production systems. The present study attempts to combine these mostly separate fields of research and agricultural knowledge. Therefore, complex model experiments were specifically designed to attempt the study of interactions between plants, heterogeneous nutrient supply in soil, bacterial and fungal organisms, and organic matter amendments to soil.

The specific objectives of the thesis were:

- to investigate the influence of AM fungi isolated from different long-term field fertilization treatments and their interaction with bacteria on plant response to soil nutrient heterogeneity caused by localized organic material amendments (Chapter 2),
- to investigate the influence of AM fungi on the plant response to soil heterogeneity caused by locally different P or N supply in split-root pots (Chapters 3 and 4), and

- to investigate the influence of AM fungi on plant response to nutrient heterogeneity caused by localized compost amendment in different growth substrates (Chapter 5).

Plants were grown for this thesis with and without AM fungi and were grown with spatially different nutrient supply (homogeneous, heterogeneous), but the total amount of nutrients supplied to plants was not varied in the experiments described in this thesis.

We hypothesised that the AM fungi often used for model experiments are very effective in the uptake of mineral P from soil, but that they do not have specific properties for the use of organic nutrient sources or nutrient patches. Further, we expected that bacterial inoculations are not effective when a bacterial community is already established in soil. Thus, such "biological fertilizers" may have a limited capacity to support good plant growth in practical agriculture. Rather, they must be part of a production system that makes wise use of organic matter, with the result of high soil fertility.

2. AVAILABILITY OF PHOSPHORUS FROM ORGANIC MATERIAL SUPPLIED IN SOIL PATCHES TO PLANTS INOCULATED WITH ARBUSCULAR MYCORRHIZAL FUNGI FROM MINERALLY OR ORGANICALLY FERTILIZED SOIL AND WITH SOIL BACTERIA

2.1 ABSTRACT

Resources in the soil are often heterogeneously distributed due to natural processes. In agricultural soils, nutrient heterogeneity can also be created by anthropogenic influence, such as directed placement of fertilizer or incorporation of crop residues or manure. Roots can respond to a heterogeneous distribution of mineral elements in the soil by root proliferation and increased nutrient uptake rates within nutrient-rich patches. Previously, these effects have often been studied with non-mycorrhizal plants in model substrates, although most plant species under natural conditions form mycorrhizal associations. The arbuscular mycorrhiza (AM) establishment and benefit for the host plant depends on the plant and AM fungal genotypes. Agricultural practices affect in the long-term fungal species composition. In the present study, sweet potato plants were grown in a low-P soil supplemented with either mineral P fertilizer or organic material (maize leaf or stem) that was homogeneously or heterogeneously distributed in the soil. The plants remained non-inoculated with mycorrhizal fungi or were inoculated with AM fungi either from a minerally or from an organically fertilized field soil, and were inoculated or not with bacteria from organically fertilized field soil. Long-term application of mineral and organic fertilizer did not have different effects on the ability of indigenous AM fungi to form mycorrhiza. Sweet potato plants benefited from the AM fungal symbiosis with respect to growth and P uptake. Plants responded to organic patches by root proliferation. Root proliferation of non-mycorrrhizal and mycorrhizal plants in organic patches was not significantly different. Plants supplied with heterogeneously distributed organic material showed higher P content and dry weight comparedto plants supplied with homogeneously distributed organic material. Regarding the organic materials, leaves tended to increase plant growth more than stem material. We conclude that mycorrhizal plants possess strategies to exploit nutrient-rich organic patches to increase their P uptake by root proliferation in the patch and by at the same time extending nutrient uptake beyond the root depletion zone outside the patches.

2.2 INTRODUCTION

Phosphorus exists in the soil in both organic and mineral form. There is much organically bound P in organic matter. However, only in mineral form P is taken up by plants (Shen et al., 2011).Microbial activity in the soil can increase plant-available soil P through the decomposition of organic matter (Prescott, 2005).

Organic matter from leaves is faster to be decomposed than from other parts of the

plants (Jian-Hui et al., 1998). The decomposition of organic matter is one of the factors causing heterogeneous nutrients distribution in soil (Emmerich et al., 2000). In agricultural soil, anthropogenic activity such as localised placement of fertilizer or incorporation of crop residue or manure also causes heterogeneous nutrient distribution (Cavagnaro et al., 2005).

Plantscan respond to heterogeneous nutrient distribution or nutrient-rich patches in soilby root proliferation, by increased nutrient uptake rate (Ma and Rengel, 2008) or by a combination of root proliferation and increased nutrient uptake rate within the patch (Zhang and George, 2008). However, someherbaceous plants which responded to nutrient-richpatches by rootproliferationdid not show an increase in specific nutrient uptake rate (Gloser et al., 2008).

Roots proliferate in the nutrient-rich patch by investing more root growth in the nutrient-rich patch than elsewhere (Hodge, 2006), so that root dry weight in the patch can be higher than root dry weight outside the patch in the same soil volume (Ma and Rengel, 2008). By local root proliferation in the nutrient-rich patch, roots can absorb more nutrients than roots growing in the nutrient-poor soil zone (George et al., 1997). In consequence, plants grown in soil with added nutrients concentrated in a patch produced more above- and belowground biomass (Lamb, et al., 2004).

Most plant species form mycorrhizal associations (Smith and Read, 1997, p.11). This association helps plants to acquire nutrients, particularly P, and hence increases plant growth. Arbuscular mycorrhizal fungi may also influence root morphological plasticity to forage for nutrients in the patch (Wijesinghe et al., 2001). Hodge and Fitter (2010) reported that AM fungi can proliferate within nutrient-rich organic patches. A possible ability of AM fungi to mineralize organic P may be due tothe excretion of phosphatase, the acidification of the hyphosphere and the association with soil bacteria (Neumann, 2007, p. 13). Thus, mycorrhizal associations may reduce the requirement for the root system to proliferate in the nutrient-rich patches (Farley and Fitter, 1999; Fitter et al., 2000, Tibbett, 2000).

However, in experiments with homogeneous nutrient supply, the growth of AM fungi can be both increased (Vaidya et al., 2008) and decreased (Ravnskov et al., 1999) by organic matter supplied to soil, depending on the nature of the material, the AM fungal genotype, and the microbial associates in the mycorrhizosphere (Linderman et al., 2003).

Long-term applications of either organic or mineral fertilizer in the soil have impacts on the diversity of AM fungi and the AM fungi efficiency to enhance plant growth. Long-term minerally fertilized soils sometimes have a lower diversity of AM fungi (Lee et al., 2008; Oehl et al., 2004) and a poorer contribution of AM fungi to host plant performance

(Johnson, 1993; Lee et al., 2008) than organically fertilized soils. In contrast, other reports showed that long-term application of cattle slurry (Cristie and Kilpatrick, 1992) or cattle manure (Ellis et al., 1992) reduced root colonization in a grass sward and soybean, respectively. Thomson et al. (1992) suggested that the differences in fungal composition due to different long term fertilization may affect the AM-mediated plant P uptake. Thus, AM fungi isolated from field plots with different long-term application of fertilizersmay not equally contribute to host nutrient uptake.

The objectives of the present study were (1) to compare the ability of AM fungi from field plots fertilized either minerally (MM) or organically (MO) throughout the last 20 years to contribute to plant P uptake from either mineral P or organic material, and (2) to assess the efficiency of mycorrhiza and/or field soil bacteria in the mobilization of P from organic material either homogeneously or heterogeneously distributed within the soil volume. This was done by comparing plants colonized by AM fungi from long-term minerally and organically fertilized field plots grown in soil supplied by either mineral fertilizer or plant material (leaf or stem) as organic fertilizer which was either homogenously or heterogeneously distributed. Further, bacteria collected from long-term organically fertilized soil were applied to detect the interaction with AM fungi to provide P from organic material for plant growth.

The purpose of the present study was to test these hypotheses:

- H1: AM fungi from organically fertilized field plots contribute better to plant nutrient uptake from organic material compared with AM fungi from minerally fertilized field plots.
- H2: AM fungi are supported by bacteria in the exploitation of organic nutrient resources in soil by increasing P availability for plant growth.
- H3: Plants have increased shoot growth when organic materials are heterogeneously distributed in soil compared with a homogeneous distribution.
- H4: Leaves as organic fertilizer lead to a stronger increase in shoot growth than stems as organic fertilizer.

2.3 MATERIALS AND METHODS

Experimental units were arranged in a fully randomized manner using a 3 x 2 x 6

factorial design where the first factor was AM fungal inoculation (AM fungi from long-term minerally fertilized soil, MM; AM fungi from long-term organically fertilized soil, MO; no AM inoculation as control, NM), the second factor was bacteria inoculation (with bacteria addition, +B; without bacteria addition, -B), and the third factor was mode of P supply to soil (addition of mineral P distributed homogeneously at low level, LP; addition of mineral P homogeneously distributed at a high level, HP;leaf material homogeneously distributed, LeHm; stem materialhomogeneously distributed, StHm; leaf material heterogeneously distributed, LeHt; stem material heterogeneously distributed, StHt). The treatment with addition of mineral P at a low level (LP) served as a control for plant growth under limited P supply. In all treatments with addition of organic material, a low amount of mineral P homogeneously distributed (as in LP) was also supplied. Each treatment combination was replicated four times.

2.3.1 PRODUCTION OF ORGANIC MATERIAL FOR SOIL AMENDMENT

Maize seeds were germinated on wet filter paper soaked with saturated $CaSO_4$ solution before they were transferred to plastic buckets (3 L; one plant per bucket) filled with nutrient solution. The nutrient solution contained 2.25 mM N (NH_4NO_3), 0.5 mM P (KH_2PO_4), 1.09 mM K (K_2SO_4 and KH_2PO_4), 2.71 mM Ca ($CaSO_4.2H_2O$), 2.71 mM S (K_2SO4 and $CaSO_4.2H_2O$), 0.06 mM Fe (Fe-EDTA), 0.02 mM B (H_3BO_3), 4 µM Mn ($MnSO_4.H_2O$), 1.84 µM Zn ($ZnSO_4.7H_2O$), 3.15 µM Cu ($CuSO_4$), and 0.27 µM Mo ($(NH_4)_6Mo_7O_{24}.H_2O$). The nutrient solution was exchanged every 4-5 days. Plants were harvested after anthesis. The biomass of the leaf blades and the stem was harvested separately. The ‘stem’ biomass included the leaf sheath, and only leaf blades were considered ‘leaf’ material. The material was applied to the soil after drying in the oven for 32 h at 65 °C and grinding in a rotation mill (ZM 100, Retsch, Germany) to the size of less than 1 mm. Nitrogen and P concentrations in both, stem and leaf, were assessed before the organic amendment was applied. Nitrogen concentrations in leaf and stem material were 23 and 19 mg per g dry weight, whereas P concentrations in leaf and stem were 6.5 mg per g dry weight.

2.3.2 INOCULUM PROPAGATION

Fresh representative soil samples were taken from either organically or minerally fertilized field plots of a long-term field fertilization experiment at the IGZ in Grossbeeren, Germany. Field soils had been fertilized with cattle manure or mineral fertilizer, respectively,

since 1989.Phosphorus concentration in the long-term minerally and organically fertilized field plots were 610 and 740 mg kg^{-1} dry soil, respectively. To propagate the AM fungi within these soil samples, 500 g of these fresh soil samples were placed in the middle of the upper layer of a pot containing 5.5 kg sieved (4 mm) C loess soil. The latter had been heated in the oven for 48 hours at 85 °C to eliminate AM fungal propagules. Five to six maize seeds were sown in each pot. Four seedlings were grown in each pot to obtain AM colonized roots as inoculum. Three pots were prepared from each type of inoculum. For non-mycorrhizal treatments, maize plants were grown in pots containing 5.5 kg sieved (4 mm) heated C loess soil without additional fresh soil. The soil in pot was supplied by 200 mg N (NH_4NO_3), 50 mg P (KH_2PO_4), 200 mg K (K_2SO_4), 100 mg Mg ($MgSO_4.7H_2O$), 10 mg Fe (Fe-EDTA), 10 mg Zn ($ZnSO_4.7H_2O$) and 10 mg Cu ($CuSO_4.5H_2O$) kg^{-1} dry soil. The inoculum was harvested eight weeks after sowing. The percentage of root length colonized by AM fungi from minerally and organically fertilized soil was 63% and 66% respectively. For non-inoculated treatments, it was 2.6%.

2.3.3 EXPERIMENTAL PLANT PREPARATION

Sweet potato (*Ipomea batatas*) motherplants were grown in nutrient solution containing 2.25 mM N (NH_4NO_3), 0.5 mM P (KH_2PO_4), 1.09 mM K (K_2SO_4 and KH_2PO_4), 2.71 mM Ca ($CaSO_4.2H_2O$), 2.71 mM S (K_2SO4 and $CaSO_4.2H_2O$), 0.06 mM Fe (Fe-EDTA), 0.02 mM B (H_3BO_3), 4 µM Mn ($MnSO_4.H_2O$), 1.84 µM Zn ($ZnSO_4.7H_2O$), 3.15 µM Cu ($CuSO_4$), and 0.27 µM Mo $(NH_4)_6Mo_7O_{24}.H_2O$). The nutrient solution was exchanged every three days. One-leaf stem cuttings with two nodes were obtained from these mother plants, and rooted in aerated 2.8 mM $CaSO_4$ solution. After the first roots had established, the $CaSO_4$ solution was replaced by the same nutrient solution as used for the motherplants, but in half strength. Plants were transferred to the experimental pots 13 days after rooting, when roots had a length of approximately 10 cm.

2.3.4 SOIL AND GROWING CONDITIONS

The experiment was conducted in a glasshouse at the Leibniz-Institute of Vegetable and Ornamental Crops, Grossbeeren (long. 13°2′E; lat. 51°22′N), Germany for nine weeks from 3 July 2007 to 10 September 2007 with a light period of approximately 14 h day/10 h night. Average light intensity was 990 µmol $m^{-2}s^{-1}$ and there was no addition of artificial light. Average air temperatures in the glasshouse during this time were 26 °C day/20 °C night and relative humidity was on average 70%.

The substrate selected to support plant growth was a C loess soil. The soil was broken up mechanically and passed through a 4 mm sieve before use. The soil was heated in the oven for 48 hours at 85 °C to eliminate AM fungal propagules. Sweet potato plants were grown in 2-L pots containing 2 kg soil with a bulk density 1.3 g dry soil cm^{-3}. To compare the relative value of root dry weight within the patches to total root dry weight in plants supplied with nutrients homogeneously and heterogeneously, two small plastic bottles with a volume 50 ml were inserted in the soil of each pot. The volume of the bottles (patches) was approximately 5% of the total volume of the bulk soil.

The bottles had two windows (6 cm^2perwindow) covered by 1-mm net, through which roots could access the inner of the bottle. For treatments with supply of mineral P homogeneously distributed at a low level (LP) and at a high level (HP) and with addition of organic material (leaf or stem) homogeneously distributed (LeHm/StHm),the bottles were filled with approximately 115 gram of a mixture of 40 µm wet sieved soil and glassbeads according to Neumann and George (2005).Nutrient contents inside and outside the bottle (patch) weresimilar for those treatments. For the treatments with supply of organic material heterogeneously distributed in soil, all organic material was placed in the bottles (patches), so that the bottles were filled with 6.92 gram dry weight of organic material (equivalent to 80 mg P and 250 mg N per bottle) and 109 gram of a mixture of wet soil and glassbeads. The organic material was mixed with the wet soil and the glassbeads before the mixture was filled in the bottle (patch). The bottles and the plant position in the experimental pot are shown in Fig. 2.1.

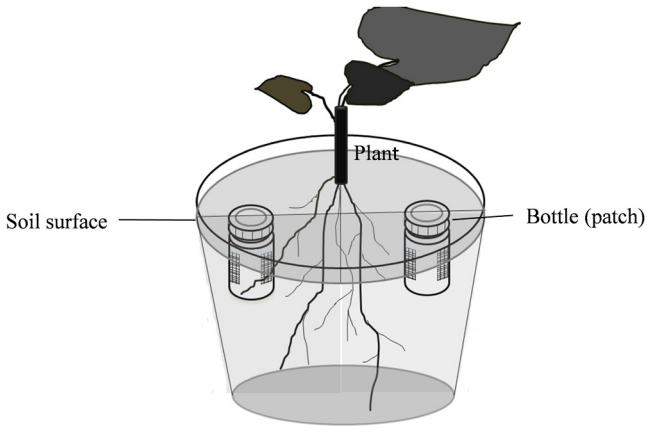

Figure 2.1: The position of plant and bottles in the experimental pot.

Different amounts of mineral N were applied in the different treatments (Tab.2.1). Extra mineral N was applied to pots without addition of organic material, to balance N supply treatments. When leaf material was applied, the soil was supplied in addition with 90 mg mineral N kg^{-1} dry soil. When stem material was applied, the soil was supplied with additional 117 mg mineral N kg^{-1} dry soil. The other treatments received 250 mg mineral N kg^{-1} dry soil (Tab. 2.1). Different rates of mineral N addition were based on the assumption of complete decomposition of the additional 6.92 gram organic material applied (from either leaf or stem) in the soil for the respective treatments, resulting in a (hypothetical) mineral N supply in all treatments of 250 mg N kg-1 dry soil.

Table 2.1: Total amount of mineral nutrients (mg per kg dry soil) supplied to the plants in bulk soil plus substrate in the patch in the different treatments with mineral fertilizer and with organic material.

Element	Applied form	Treatment			
		Low mineral P supply	High mineral P supply	Supply of leaf material (Le)	Supply of stem material (St)
N	NH_4NO_3	250	250	90	117
P	KH_2PO_4	35	80	35	35
K	K_2SO_4 and KH_2PO_4	200	200	200	200
Mg	$MgSO_4.7H_2O$	100	100	100	100
Fe	Fe-EDTA	10	10	10	10
Zn	$ZnSO_4.7H_2O$	10	10	10	10
Cu	$CuSO_4.5H_2O$	10	10	10	10

For P, no extra additions were made to pots without addition of organic material. The water content of the soil was adjusted to approximately 17% w/w after the plants were inserted. Water loss from the pots was estimated gravimetrically, and was replaced by deionized water every two days.

2.3.5 PLANT INOCULATION WITH ARBUSCULAR MYCORRHIZAL FUNGI AND SOIL BACTERIA

In the present experiment, the AM mycorrhizal inoculum consisted of root fragments colonized by AM fungi either from long-term minerally or organically fertilized soil.Before

they were used as inoculum, AM colonized and uncolonized fragments were soaked in Chlorix 0.005 % for 30 s, Gentamycin 0.01 % for 3-5 min, and Streptomycin 0.02 % for 3-5 min to reduce the number of attached soil bacteria. Each pot was inoculated either with approximately 2g fresh root fragments colonized by AM fungi for mycorrhizal treatments or with 2 g fresh root fragments uncolonized by AM fungi for non-mycorrhizal treatments. Inoculum was placed in the vicinity of sweet potato plant roots when at planting.

Bacteria were extracted from 2 kg fresh soil of the long-term organically fertilized field plots. Portions of 50 g fresh soil were filtered with 100 ml deionized water through filter paper (Rotilabo R Faltenfilter 50s, Carl Roth). Each pot of the bacteria inoculated treatments (+B) received 40 ml of this aqueous filtrate. The non-bacterial treatments (-B) received the same amount of autoclaved filtrate.

2.3.6 HARVEST AND ANALYSIS OF PLANT AND ARBUSCULAR MYCORRHIZAL FUNGAL MATERIAL

After nine weeks of growth, sweet potato shoots, roots (without tubers) and tubers were harvested separately. The roots and tubers in the bulk soil were washed from soil with tap water. Representative fresh samples of roots (approximately 1 g fresh weight; without tubers) from the bulk soil were taken to estimate the extent of AM fungal root colonization. Roots (including tubers) and residue of organic material were also separated from the wet sieved soil in the bottle (patch), and then roots (including tuber) were separated from organic material. A fresh representative sample of root (without tubers) from the bottle was taken to estimate the extent of AM fungal root colonization. The rest of roots (without tuber) and the organic material from the bottle were submitted to freeze drying. Shoot, root, and tuber dry weights in the bulk soil were determined after drying at 80 °C for 48 h.

Total plant dry weight (DW) was determined by adding shoot, root and tuber DW of each plant. The shoot/root ratio was determined by shoot DW divided by total root DW from outside and inside the patch. Relative value of root DW in the patches to total root DW was determined by root DW in the patches divided by total root DW of each plant.

The contribution of AM fungi to plant growth was calculated based on the change in plant biomass that results from symbiosis. The equation of the contribution of AM fungi to plant growth was adapted from the equation of plant responsiveness to AM colonization according to Smith and Smith (2011). This equation for the contribution of AM fungi to plant growth is 100 x (AM-NM)/NM. In this equation, AM and NM refer to biomass of mycorrhizal (AM) and non-mycorrhizal (NM) plants.

To assess the AM colonized root length, the designated sub-samples from roots in the pot as well as from roots in the bottles (patches) were cleared and stained with trypan blue in lactic acid according to Philips and Hayman (1970). Approximately 200 root intersections were counted for mycorrhizal colonization assessment by a gridline intersection procedure according to Giovannetti and Mosse (1980). The AM colonized root length is given in percent of the total root length.

To analyse nutrient concentrations in the plant tissue (shoot and root), dried shoot and root (without tuber) material from each plant was ground into fine powder. Shoot material was ground in a Retsch ZM mill and root material was ground in a Fritsch Pulverisetter mill. A 0.5 gram sample of ground shoot was transferred to a 25 ml beaker glass and ashed in the oven for 4 hours at 500 °C. Thereafter, the sample was cooled, 2.5 ml of HNO_3 1:2 was added, and the sample was then heated on a hot plate until the dense white fumes disappeared and a transparent to white content was left. The sample was then cooled and 2.5 ml of HCl 1:2 added, then about 10 ml of warm double distillated water was added, and the sample was then stirred with a glass stick. Then, samples were transferred to a 25-ml conical flask and two pieces of carborundum stones added. Double distillated water was added until half of the volume of the conical flask. The sample was then boiled on a hot plate, cooled and double distillated water added until the 25 ml mark. The sample was thereafter transferred to a storage bottle using filter paper (Whatman filter paper circles 593/3).

For root P analysis, 200 mg of ground root material was transferred to MF vessels of a microwave system and 5 ml of HNO_3 60% and 2 ml H_2O_2 30%were added. The samples were kept for 20 minutes without covering the vessels, digested in a microwave, transferred to a 25 ml conical flask and made up to volume of 25 ml with double distillated water, and then transferred to a storage bottle using filter paper (Whatman filter paper circles 593/3). Phosphate concentrations in these filtrates were measured by an EPOS Analyzer 5060. The P content of eithershoot or root was calculated by multiplying their biomass with their P concentration. There was no P or N analysis for tuber material.

For N analysis, the ground shoot and roots were decomposed by dry oxidation (Dumas method). The extraction of N was done by explosive combustion in an oxygen enriched helium atmosphere surrounded by a copper oxide filled pipe at a temperature of 980 °C. The resulting gas mix was submitted to a gas-phase chromatograph where N could be quantified in a thermal conductivity tube. An associated processor calculatedthe percentage of N measured (Elementar Vario EL). The N content of either shoot or roots was calculated by multiplying their biomass with their N concentration.

2.3.7 STATISTICAL ANALYSIS

The experiment was a completely randomized design with four replicates per treatment. Treatment effects were statistically analyzed by SPSS (SPSS 15, SPSS Inc. Chicago, USA). A multivariate ANOVA was calculated, considering all three experimental factors and their different levels (AM fungal inoculation: MM, MO, NM; bacteria inoculation: +B, -B; mode of P supply to soil: LP, HP, LeHm, StHM, LeHt, StHt). For some parameters, Five-, Four- or Three-Way ANOVA tables were calculated to test, for example, contrasts between the two levels of mineral P supply (LP vs. HP) or between leaf and stem supply material (Le vs. St). The ANOVA tables with the respective degrees of freedom are presented in this chapter for selected parameters. Duncan Multiple Range Tests were conducted to determine the differences between treatment means when appropriate. For all tests, differences were considered significant when $P<0.05$. For belowground measurements, in addition tests were made for significance between observations outside (OP) and inside (IP) the patches (patch local effect) in the respective treatments.

2.4 RESULTS

2.4.1 TOTAL PLANT DRY WEIGHT

Total plant DWwas increased in response to the higher level of mineral P supply (HP vs.LP; Fig. 2.2 and Tab. 2.2.A). The total plant DW of plants supplied with the higherlevel of mineral P (HP) was not significant different from total plant DW of plants supplied with organic material (leaf or stem) heterogeneously distributed (Ht) (HP vs. Ht; Fig. 2.2 and Tab. 2.2.C). However, total plant DW of plants supplied with the higherlevel of mineral P (HP) was higher than that of plants supplied with organic material homogeneously distributed (Hm) (HP vs. Hm; Fig.2.2 and Tab. 2.2.D). The total plant DW of plants supplied with organic material heterogeneously distributed (Ht) was higher than that of plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.2. and Tab. 2.2.B).

The plant DW responded positively to colonization by AM fungi from both minerally and organically fertilized field plots in all supply treatments (Fig. 2.2 and Tabs.2.2.A, 2.2.B). The contribution of AM fungi from both minerally and organically fertilized field plots to increase plant DW was highest in plants supplied with the lower level of mineral P (LP) (Fig. 2.2 and Tab.2.2.E) whileplants supplied with the higher level of mineral P (HP) showed the

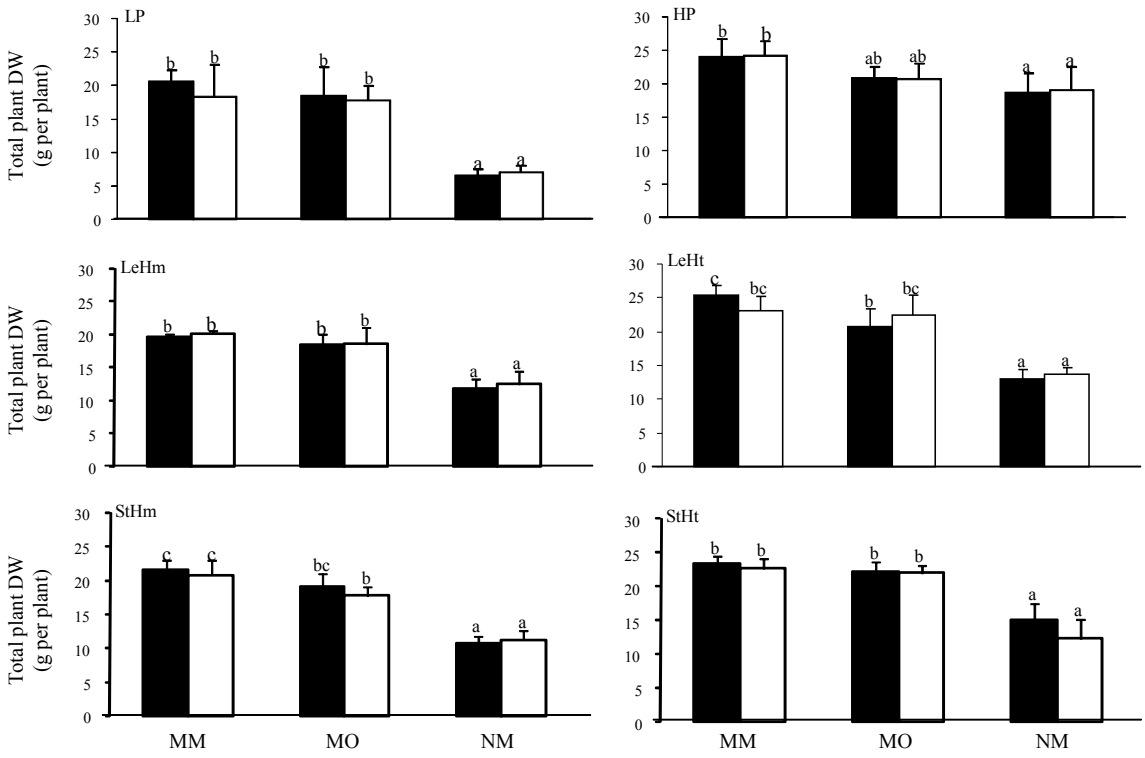

Figure 2.2: Total plant DW of sweet potato plants. The plants were either not inoculated with AM fungi (NM) or inoculated with AM fungi from minerally (MM) or organically (MO) fertilized field plots and either not inoculated with bacteria (-B, white bar) or inoculated with bacteria (+B, black bar). The soil was supplied with mineral P at low level (LP), with mineral P at high level (HP), with leaf material homogeneously distributed (LeHm) or with stem material homogenously distributed (StHm), with leaf material heterogeneously distributed (LeHt) or with stem material heterogeneously distributed (StHt). Values are means and standard deviation (SD) of four replicates of each treatment. Bars for each supply treatment with the same letter are not significantly different ($P<0.05$).

lowest AM contribution to increase plant DW (Fig. 2.2 and Tab.2.2.E). The contribution of AM fungi from minerally fertilized field plots (MM) to plant dry weight was larger compared with that of AM fungi from organically fertilized field plots (MO)(MM vs. MO; Fig. 2.2. and Tabs.2.2.A, 2.2.B). There was no significant effect of the bacteria application (+B vs. –B) or of the type of organic material (Le vs. St) on total plant DW (Fig. 2.2 and Tabs. 2.2.A, 2.2 B).

2.4.2 SHOOT DRY WEIGHT

Shoot DW was increased in response to the higherlevel of mineral P supply (HP vs. LP; Fig. 2.3 and Tab. 2.2.A). The shoot DW of plants supplied with the higher level of mineral P (HP)were not significantly different from the shoot DW of plants supplied with leaf material heterogeneously distributed (LeHt) and were significantly higher than that of plants supplied with stem material heterogeneously distributed (StHt) (HP vs Ht; Fig. 2.3 and Tab. 2.2.C).

Table 2.2A: A Three-Way ANOVA was performed on data obtained for the treatments that received mineral P supply only. The tested treatments were level of mineral P supply (LP, HP), AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these mains factor is indicated by a star. Significant interactions ($P<0.05$) are also given. In case the ANOVA indicated a significant effect of AM inoculation, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different AM inoculation treatments differ. The results are shown in last row.

Treatments	df	Total plant DW	Shoot DW	Shoot/root ratio	Relative value of root DW in the patches to total root DW
Main factors:					
Mineral P supply	1	*	*	*	*
AM inoculation	2	*	*	*	*
Bacteria inoculation	1	ns	ns	ns	ns
Interactions:					
Mineral P supply x AM inoculation	2	*	*	*	ns
DMRT for AM inoculation		MM > MO > NM	MM,MO > NM	MM, MO > NM	MM, MO > NM

Table 2.2.B: A Four-Way ANOVA was performed on data obtained for the treatments that were supplied with organic material. The tested treatments were AM inoculation (MM, MO, NM), bacteria inoculation (+B, -B), type of organic material (Le, St) and distribution of organic material (Hm, Ht). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interactions ($P<0.05$) are also given. For further explanation see Tab. 2.2.A.

Treatments	df	Total plant DW	Shoot DW	Shoot/root ratio	Relative value of root DW in the patches to total roots DW
Main factors:					
AM inoculation	2	*	*	ns	ns
Bacteria inoculation	1	ns	ns	ns	ns
Type of organic material (OM)	1	ns	*	ns	*
Distribution of organic material (OM)	1	*	*	*	*
Interactions:					
AM inoculation x OM distribution	2	ns	ns	*	ns
OM type x OM distribution	1	ns	*	ns	*
Bacteria inoculation x OM type x OM distribution	1	ns	ns	ns	ns
DMRT for AM inoculation		MM > MO > NM	MM > MO > NM	-	-

Table 2.2.C: A Three-Way ANOVA was performed on data obtained for the treatments that were supplied with mineral P in high level (HP) and organic material heterogeneously distributed (Ht). The tested treatments were high level of P supply (HP, LeHt, StHt), level of AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B), A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interactions ($P<0.05$) are also given. In case the ANOVA indicated a significant effect of either high level P supply or AM inoculation, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different high level P supply or AM inoculation treatments differ. The results are shown in the last rows.

Treatments	df	Total plant DW	Shoot DW	Shoot/root ratio	Relative value of root DW in the patches to total root DW
Main factors:					
High level of P supply	2	*	*	ns	*
AM inoculation	2	*	*	ns	ns
Bacteria inoculation	1	ns	*	ns	ns
Interactions:					
High level of P supply x AM inoculation	4	*	ns	ns	*
DMRT for high level of P supply		-	LeHt, HP > StHt	-	HP, LeHt > StHt
DMRT for AM inoculation		MM >MO > NM	MM > MO > NM	-	-

Table 2.2.D: A Three-Way ANOVA was performed on data obtained for the treatments that were supplied with mineral P in high level (HP) and organic material homogeneously distributed (Hm). The tested treatments were high level of P supply (HP, LeHm, StHm), level of AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interactions ($P<0.05$) are also given. For further explanation see Tab. 2.2.C.

Treatments	df	Total plant DW	Shoot DW	Shoot root ratio	Relative value roots DW in the patches to total root DW
Main factors:					
High level of P supply	2	*	*	*	*
AM inoculation	2	*	*	*	ns
Bacteria inoculation	1	ns	ns	ns	ns
Interactions:					
High level of P supply x AM inoculation	4	*	ns	ns	ns
DMRT for high level of P supply		HP > LeHm, StHm	HP > LeHm, StHm	HP > LeHm, StHm	HP > LeHm, StHm
DMRT for AM inoculation		MM > MO > NM	MM, MO > NM	MM > MO, NM	-

Table 2.2.E: A Three-Way ANOVA was performed on data obtained for the treatments that were supplied with all modes of P supply to soil. The tested treatments were all modes of P supply (LP, HP, LeHm, StHm, LeHt, StHt), AM inoculation (MM, MO) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these main factors is indicated by a star. In case the ANOVA indicated a significant effect of mode of P supply, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different high level P supply treatments differ. The results are shown in last row.

Treatments	df	Contribution of mycorrhiza to total plant DW
Main factors:		
Mode of P supply	5	*
AM inoculation	1	*
Bacteria inouclation	1	ns
Interaction:		
Mode of P supply x AM inoculation	28	-
DMRT for mode of P supply		LP > StHm,StHt,LeHt,LeHm > HP

The shoot DW of plants supplied with the higher level of mineral P (HP) was higher than that of plants supplied with organic material (leaf or stem) homogeneously distributed (Hm) (HP vs. Hm; Fig. 2.3 and Tab. 2.2.D). The shoot DW of plants supplied with organic material heterogeneously distributed (Ht) was higher than that of plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.3 and Tab. 2.2.B). Plants supplied with leaf material (Le) had the higher shoot DW than plants supplied with stem material (St) particularly when organic material was heterogeneously distributed (Ht) (Le vs. St; Fig. 2.3 and Tab. 2.2.B).

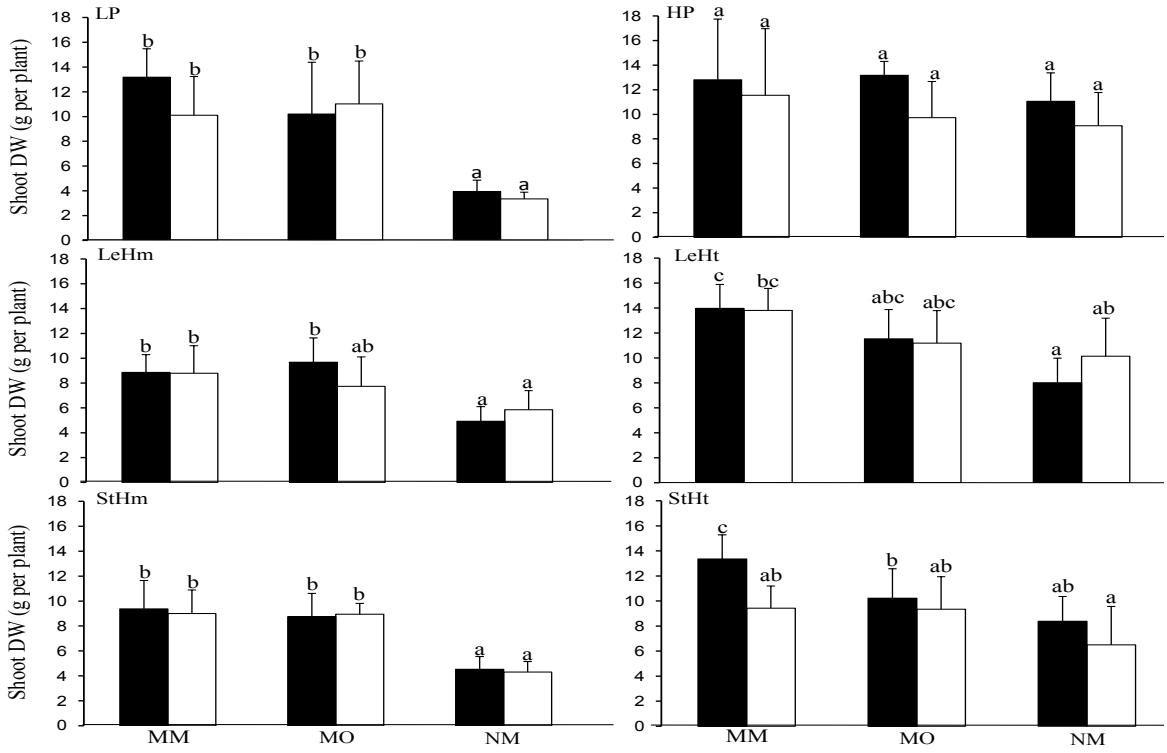

Figure 2.3: Shoot DW of sweet potato plants. For further explanation see Fig. 2.2.

The shootDW of plants supplied with the lower level of mineral P (LP) and organic material either homogeneously (Hm) or heterogeneously (Ht) distributed responded positively to AM colonization (Fig. 2.3 and Tabs. 2.2.A, 2.2.B), while shoot DW of plants supplied with the higher level of mineral P did not response positively to AM colonization (Fig. 1.2). The contribution of AM fungi from minerally fertilized field plots (MM) to shoot DW was larger compared with that of AM fungi from organically fertilized field plots (MO) in plants supplied with organic material (MM vs. MO; Fig. 2.3 and Tab. 2.2 B). There was no effect of bacteria (+B vs. –B) on shoot DW (Fig. 2.3 and Tabs. 2.2.A, 1.1.B).

2.4.3 SHOOT/ROOT RATIO

In non-mycorrhizal plants, the shoot/root ratio was increased in response to the higher level of mineral P supply (HP vs. LP; Fig. 2.4; Tab. 2.2.A). The shoot/root ratioof plants supplied with the higher level of mineral P (HP) was not significantly different from the shoot/root ratio of plants supplied with organic material (leaf or stem) heterogeneously distributed (Ht) (HP vs. Ht; Fig. 2.4 and Tab. 2.2.C). The shoot/root ratio of plants supplied with organic material heterogeneously distributed (Ht) was higher than that of plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.4 and Tab. 2.2.B).

Application of AM fungi increased the shoot/root ratio at low mineral P supply (LP) (Fig. 2.4 and Tab. 2.2.A). The AM fungi from minerally (MM) and from organically (OM) fertilized field plots did not differ significantly in their effect on the shoot/root ratio (MM vs. OM; Fig. 2.4 and Tabs. 2.2.A, 2.2.B). Mycorrhiza fungal treatments had no significant effect on the shoot/root ratio of plants supplied either with the higher level of mineral P (HP) or with organic material heterogeneously distributed (Ht) (Fig. 2.4 and Tabs. 2.2.B, 2.2.C). There was no effect of bacteria (+B vs. –B) or the type of organic material applied (Le vs. St.) on shoot/root ratio (Fig. 2.4 and Tabs. 2.2.A, 2.2.B).

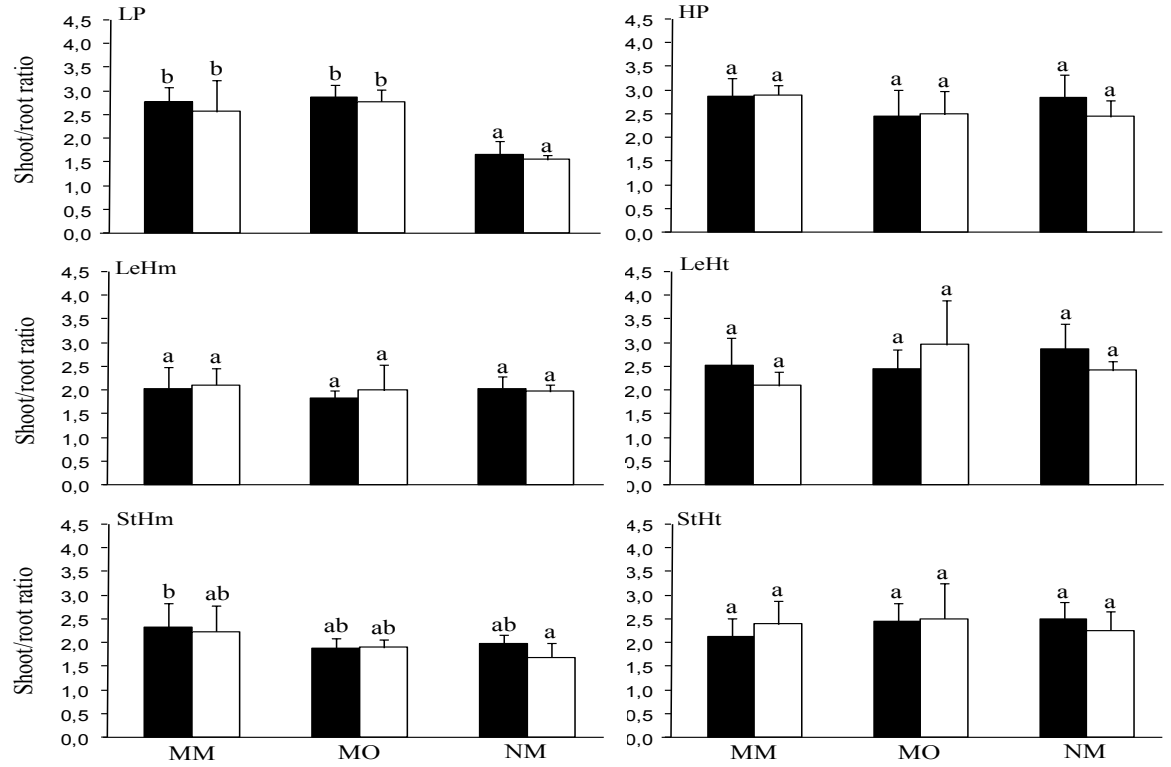

Figure 2.4: Shoot/root ratio of sweet potato plants. For further explanation see Fig. 2.2.

2.4.4 RELATIVE VALUE OF ROOT DRY WEIGHT IN THE PATCHES TO TOTAL ROOT DRY WEIGHT

The relative value of root DW in the patches to total root DWwas higher in plants supplied with organic material heterogeneously distributed(Ht) than in plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.5 and Tab. 2.2.B). Plants supplied with leaf material (Le) had higher relative value of root DW in the patches to total root DW than plants supplied with stem material (St) (Le vs. St; Fig. 2.5 and Tab. 2.2.B). Neither mycorrhizal colonization nor bacteria inoculation (+B vs –B) had any significant effect on the relative value of root DW in the patches to total root DW (Fig. 2.5 and Tab. 2.2.B).

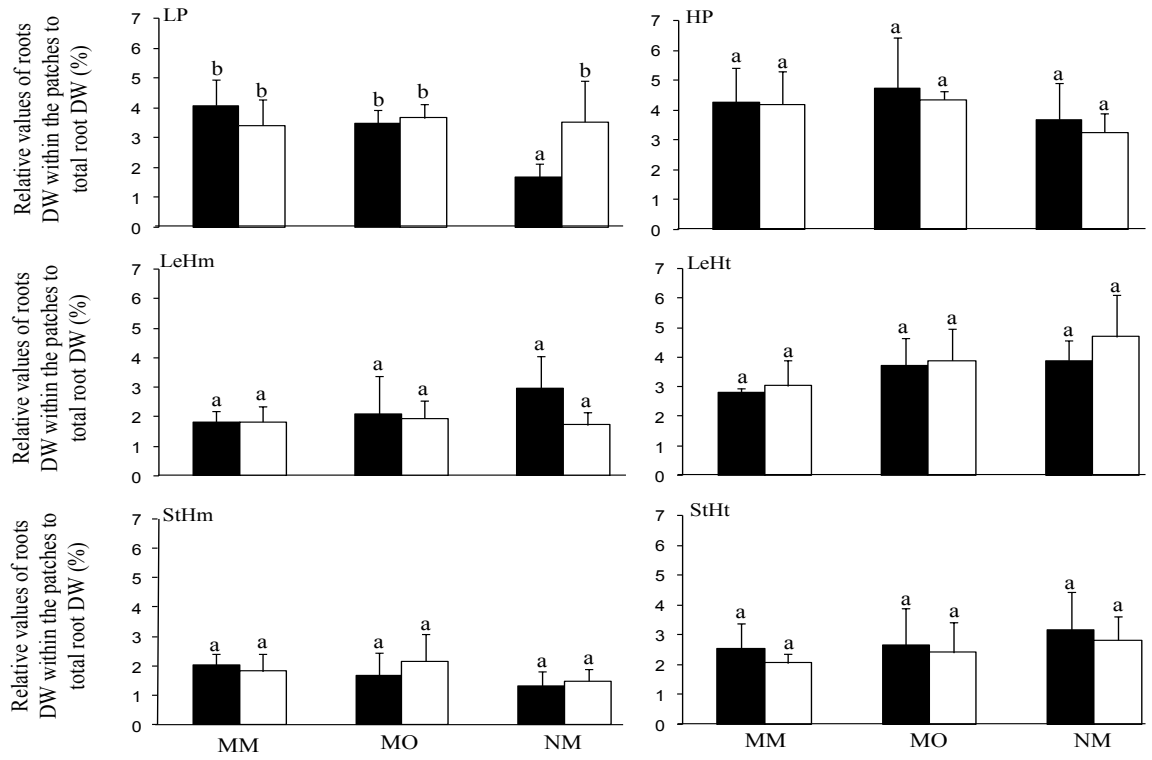

Figure 2.5: Relatives value of root DW within the patches to total root DW. For further explanation see Fig. 2.2.

2.4.5 COLONIZATION BY ARBUSCULAR MYCORRHIZAL FUNGI OUTSIDE AND INSIDE THE PATCHES

In non-mycorrhizal plants, the rates of AM root colonization outside the patches were 0-7% (data not shown), while in mycorrhizal plants the rates of AM root colonization outside the patches and inside the patches were 34-75% and 20-72%, respectively (Fig. 2.6). In mycorrhizal plants, the rate of AM root colonization both outside and inside the patches was decreased with the higher level of mineral P supply (HP vs. LP; Fig. 2.6 and Tab. 2.3.A). There was no significant difference between AM colonization outside and inside the patches atthe lower level of mineral P supply (LP), atthe higher level of mineral P supply (HP) and in the organic material (leaf or stem) homogeneously distributed treatments (OP vs. IP; Fig. 2.6 and Tabs. 2.3.A, 2.3.B). Plants supplied with organic material heterogeneously distributed (Ht) had distinctly lower AM colonization rates inside the patches than outside the patches (OP vs. IP; Fig. 2.6 and Tab. 2.3.B).

Table 2.3.A: A Four-Way ANOVA was performed on data obtained for the treatments that received mineral P supply only. The tested treatments were level of mineral P supply (LP, HP), AM inoculation (MM, MO), bacteria inoculation (+B, -B), and patch local effect (OP, IP). A significant effect ($P<0.05$) effect of the main factors is indicated by a star.

Treatments	df	AM colonization
Main factors:		
Mineral P supply	1	*
AM inoculation	1	ns
Bacteria inoculation	1	ns
Patch local effect	1	ns

Table 2.3.B: A Five-Way ANOVA was performed on data obtained for the treatments which were supplied with organic material. The tested treatments were AM inoculation (MM, MO), bacteria inoculation (+B, -B), type of organic material (Le, St), distribution of organic material (Hm, Ht), and patch local effect (OP, IP). A significant effect ($P<0.05$) effect of the main factors is indicated by a star. Significant interactions ($P<0.05$) are also given.

Treatments	df	AM colonization
Main factors:		
AM inoculation	1	ns
Bacteria inoculation	1	ns
Type of organic material (OM)	1	*
Distribution of organic material (OM)	1	*
Patch local effect	1	*
Interactions:		
AM inoculation x OM type	1	*
Bacteria inoculation x OM distribution	1	*
AM inoculation x bacteria inoculation x patch local effect	1	*
OM distribution x patch local effect	1	*

Table 2.3.C: A Four-Way ANOVA was performed on data obtained for the treatments that were supplied with mineral P at high level (HP) and organic material heterogeneously distributed (Ht). The tested treatments were high level of P supply (HP, LeHt, StHt), AM inoculation (MM, MO), bacteria inoculation (+B, -B) and patch local effect (OP, IP). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction ($P<0.05$) is also given.

Treatments	df	AM colonization
Main factors:		
High level of P supply	2	ns
AM inoculation	1	ns
Bacteria inoculation	1	ns
Patch local effect	1	*
Interactions:		
High level of P supply x patch local effect	2	*

Table 2.3.D: A Four-Way ANOVA was performed on data obtained for the treatments that were supplied with mineral P at high level (HP) and organic material homogeneously distributed (Hm). The tested treatments were high level of P supply (HP, LeHm, StHm), AM inoculation (MM, MO), bacteria inoculation (+B, -B), and patch local effect (OP, IP). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction ($P<0.05$) is also given. In case the ANOVA indicated a significant effect of high level P supply, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different high level P supply treatments differ. The results are shown in the last row.

Treatments	df	AM colonization
Main factors:		
High level of P supply	2	*
AM inoculation	1	ns
Bacteria inoculation	1	ns
Patch local effect	1	*
Interactions:		
High level of P supply x AM inoculation x patch local effect	2	*
DMRT for high level of P supply		HP > LeHm > StHm

The AM colonization outside the patches in plants supplied with organic material heterogeneously distributed (Ht) was higher than in plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.6 and Tab. 2.3.B). Plants supplied with leaves as organic material (Le) had higher AM colonization rates than plants supplied with stem material (St) (Le vs. St; Fig. 2.6 and Tab. 2.3.B). Neither bacteria (+B vs. –B) nor the origin of mycorrhizal fungi (MM vs. MO) had an effect on the rate of AM root colonization outside and inside the patch (Fig. 2.6 and Tab. 2.3.B). The rate of AM root colonization in organic matter rich patches was lower than the rate of AM root colonization at the higher mineral P supply (Fig. 2.6 and Tab. 2.3.C). The rate of AM root colonization was higher in plants supplied with the higher mineral P supply than in plants supplied with organic matter distributed homogeneously (Fig. 2.6 and Tab. 2.3.D).

2.4.6 TOTAL PLANT PHOSPHORUS CONTENT

The higher level of mineral P supply (HP) increased total plant P content compared to the lower level of mineral P supply (LP) (HP vs LP; Fig. 2.7 andTab. 2.4.A). The total plant P content in plants supplied with the higher level of mineral P (HP) was not significantly different fromthe total plant P content in plants supplied with leaf material heterogeneously distributed (LeHt) and was significantly higher than that in plants supplied with stem material heterogeneously distributed (HP vs. StHt; Fig. 2.7 and Tab. 2.4.C). Plants supplied with the higher level of mineral P (HP) had higher total plant P content than plants supplied with

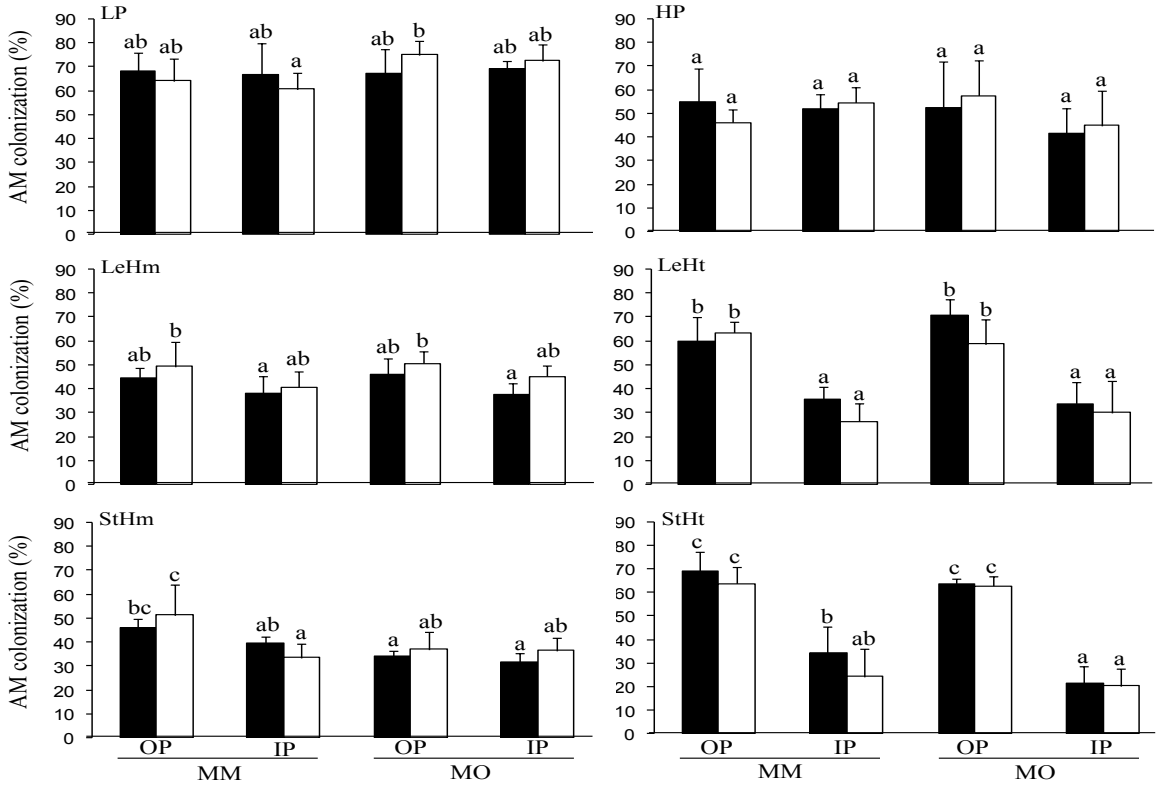

Figure 2.6: Rate of AM colonization outside (OP) an inside (IP) the patches (patch local effect). The plants were either inoculated with AM fungi from minerally (MM) or organically (MO) fertilized field plots or either not inoculated with bacteria (-B, white bar) or inoculated with bacteria (+B, black bar). The soil was supplied with mineral P at low level (LP), with mineral P at high level (HP), with leaf material homogeneously distributed (LeHm) or with stem material homogeneously distributed (StHm), with leaf material heterogeneously distributed (LeHt) or with stem material heterogeneously distributed (StHt). Values are means and SD of four replicates of each treatment. Bars for each supply treatment with the same letter are not significantly different ($P<0.05$).

organic material (leaves or stem) homogeneously distributed (Hm) (HP vs. Hm; Fig. 2.7 and Tab. 2.4.D). The total P content in plants supplied with organic material heterogeneously distributed (Ht) was higher than in plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.7 and Tab. 2.4.B).

Leaves as organic material (Le) increased plant P content to a greater extent compared with stem material (St) (Le vs. St; Fig. 2.7 and Tab. 2.4.B). Total P content was drastically increased in response to colonization with AM fungi in plants supplied either with mineral P or with organic material (Fig. 2.7 and Tabs. 2.4.A, 2.4.B). Plants colonized with AM fungi from minerally fertilized field plots (MM) had a higher P content compared with plants colonized by AM fungi from organically fertilized field plots (OM) (MM vs. MO). This

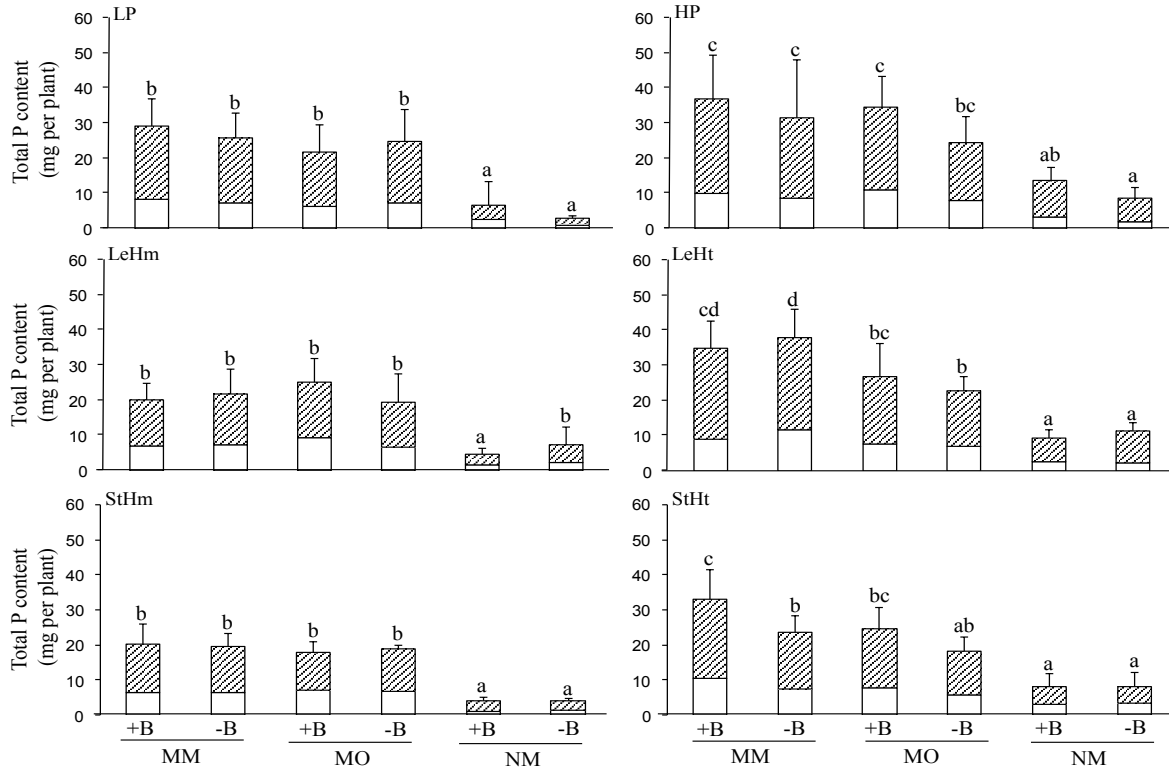

Figure 2.7: Total P content of shoot (diagonally hatched bar) and roots (white bar). The plants were either not inoculated with AM fungi (NM) or inoculated with AM fungi from minerally (MM) or organically (MO) fertilized field plots and either not inoculated with bacteria (-B) or inoculated with bacteria (+B). The soil was supplied with mineral P at low level (LP), with mineral P at high level (HP), with leaf material homogeneously distributed (LeHm) or with stem material homogeneously distributed (StHm), with leaf material heterogeneously distributed (LeHt) or with stem material heterogeneously distributed (StHt). Values are means and SD of four replicates of each treatment. Bars for each supply treatment with the same letter are not significantly different for total plant P content.

difference was expressed mainly when plants were supplied with organic material (leaf or stem) heterogeneously distributed (Ht) (Fig. 2.7 and Tab. 2.4.B). The effect of bacteria inoculation (+B vs. –B) on total plant P content was not significant.

Table 2.4.A: A Three-Way ANOVA was performed on data obtained for the treatments that received mineral P supply only. The tested treatments were level of mineral P supply (LP, HP), AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction ($P<0.05$) is also given. In case the ANOVA indicated a significant effect of AM inoculation, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different AM inoculation treatment differ. The results are shown in last row.

Treatments	df	Total P content	Total N content
Main factors:			
Mineral P supply	1	*	ns
AM inoculation	2	*	*
Bacteria inoculation	1	ns	*
Interaction:			
Mineral P supply x AM inoculation	2	ns	*
DMRT for AM inoculation		MM, MO > NM	MM, MO > NM

Table 2.4.B: A Four-Way ANOVA was performed on data obtained for the treatments that were supplied with organic material. The tested treatments were AM inoculation (MM, MO, NM), bacteria inoculation (+B, -B), type of organic material (Le, St) and distribution of organic material (Hm, Ht). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction ($P<0.05$) is also given. For further explanation see Tab. 2.4.A.

Treatments	df	Total P content	Total N content
Main factors:			
AM inoculation	2	*	*
Bacteria inoculation	1	ns	ns
Type of organic material (OM)	1	*	*
Distribution of organic material (OM)	1	*	*
Interactions:			
AM inoculation x OM distribution	2	*	*
DMRT for AM inoculation		MM > MO > NM	MM, MO > NM

Table 2.4.C: A Three-Way ANOVA was performed on data obtained for the treatments that were supplied with mineral P at high level (HP) and organic material heterogeneously distributed (Ht). The tested treatments were high level of P supply (HP, LeHt, StHt), AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction ($P<0.05$) is also given. In case the ANOVA indicated a significant effect of either high level P supply or AM inoculation, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different either high level P supply or AM inoculation treatments differ.

Treatments	df	Total P content	Total N content
Main factors:			
High level of P supply	2	*	*
AM inoculation	2	*	*
Bacteria inoculation	1	*	ns
Interactions:			
High level of P supply x Bacteria inoculation	2	ns	*
DMRT for high level of P supply		LeHt, HP > StHt	HP > LeHt > StHt
DMRT for AM inoculation		MM > MO > NM	MM, MO > NM

Table 2.4.D: A Three-Way ANOVA was performed on data obtained for the treatments that were supplied with mineral P in high level (HP) and organic material homogeneously distributed (Hm). The tested treatments were high level of P supply (HP, LeHm, StHm), AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction ($P<0.05$) is also given. For further explanation see Tab. 2.4.C

Treatments	df	Total P content	Total N content
Main factors:			
High level of P supply	2	*	*
AM inoculation	2	*	*
Bacteria inoculation	1	ns	*
Interactions:			
High level of P supply x bacteria inoculation	2	ns	*
DMRT for high level P supply		HP > LeHm, StHm	HP > StHm, LeHm
DMRT for AM inoculation		MM, MO > NM	MO, MM > NM

2.4.7 TOTAL PLANT NITROGEN CONTENT

The total plant N content was increased by inoculation with AM fungi (Fig. 2.8 and Tab. 2.4.A). There was no significant difference in total plant N content between mycorrhizal plants supplied with the lower and the higher mineral P level (HP vs LP; Fig. 2.8 andTab. 2.4.A). Plants supplied with the higher level of mineral P (HP) had a higher total N content than plants supplied with organic material (leaf or stem) either heterogeneously or homogeneously distributed (HP vs. Ht, Hm; Fig.2.8 and Tabs. 2.4.C, 2.4.D). The N content of plants supplied with organic materialheterogeneously distributed (Ht) was higher than that of plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig.

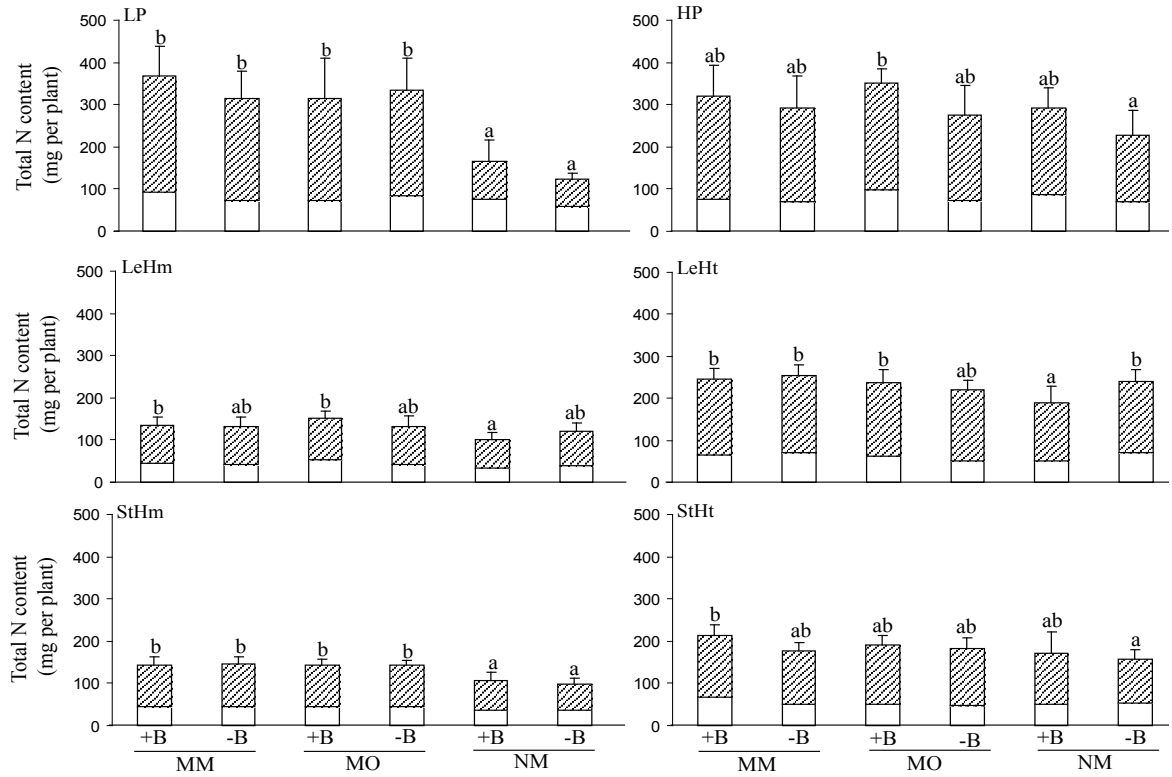

Figure 2.8: Total N content of shoot (diagonally hatched bar) and roots (white bar). For further explanation see Fig. 2.7.

2.8 and Tab. 2.4.B).

The N content of plants supplied with leaf material (Le) was higher than that of plants supplied with stem material (St) (Le vs. St; Fig. 2.8 and Tab. 2.4.B). The AM fungi increased plant N content especially in plants supplied with the lower amount of mineral P or with organic material heterogeneously distributed (Ht) (Fig. 2.8 and Tabs. 2.4.A, 2.4.B). There was no significant difference between AM fungi from minerally and organically fertilized field plots in the effect on total plant N content (MM vs. MO; Fig. 2.8 and Tabs. 2.4.A, 2.4.B).The effect of bacteria (+B vs –B) on total plant N content was not significant when plantswere supplied with organic material heterogeneously distributed (Fig. 2.8 and Tab. 2.4.C).

2.4.8 PHOSPHORUS CONCENTRATIONS IN THE SHOOT AND IN THE ROOT

Thehigher level of mineral P supply (HP) had no significant effect on P concentrations in the shoot compared with the lower level of mineral P supply (LP) (HP vs.

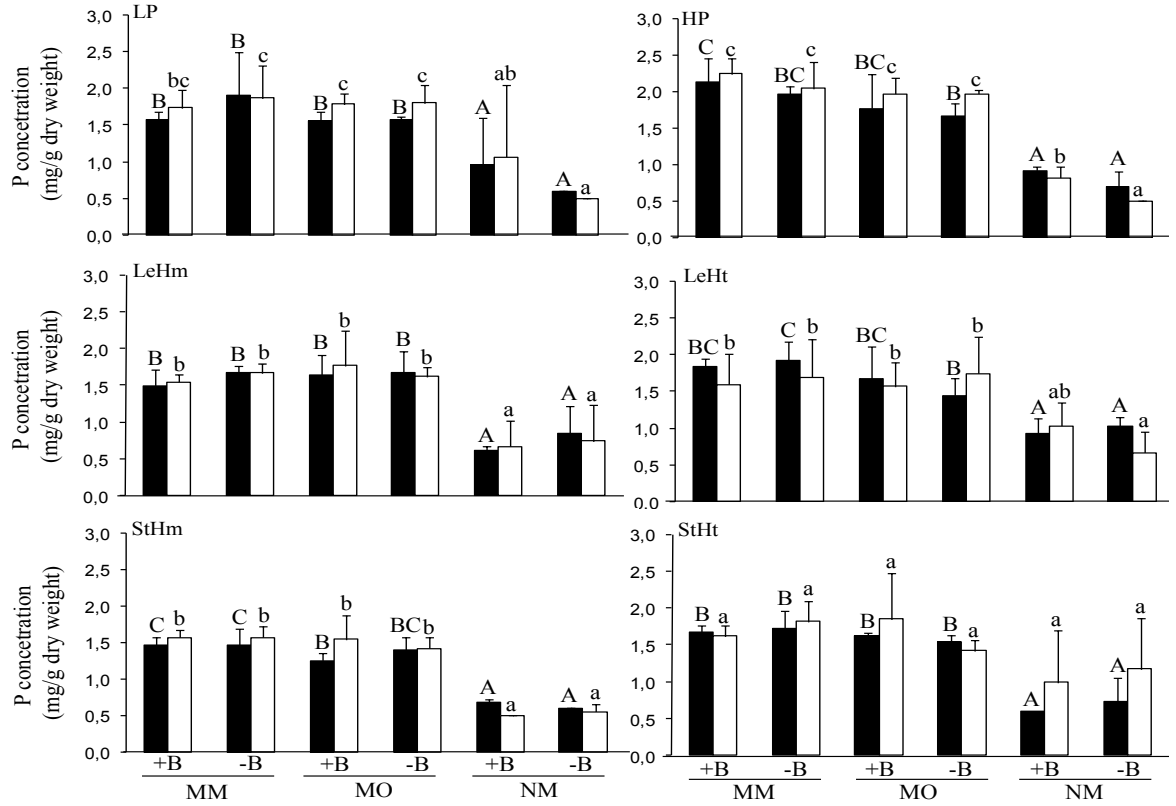

Figure 2.9: P concentrations in the shoot (black bar) and in the roots (white bar). The plants were either not inoculated with AM (NM) or inoculated with AM fungi from minerally (MM) or organically (MO) fertilized field plots or either not inoculated with bacteria (-B) or inoculated with bacteria (+B). The soil was supplied with mineral P at low level (LP), with mineral P at high level (HP), with leaf material homogeneously distributed (LeHm) or with stem material homogeneously distributed (StHm), with leaf material heterogeneously distributed (LeHt) or with stem material heterogeneously distributed (StHt). Values are means and SD of four replicates of each treatment. Bars for each supply treatment with the same letter are not significantly different ($P<0.05$).

LP; Fig. 2.9, Tab. 2.5.A). Shoot P concentrations of plants supplied with the higher level of mineral P supply (HP) were not significantly different from shoot P concentrations of plants supplied with leaf material heterogeneously distributed (LeHt) and were significantly higher than those of plants supplied either with stem material heterogeneously distributed (StHm) or organic material (leaf or stem) homogeneously distributed (Hm) (HP vs StHt, Hm; Fig. 2.9 and Tabs. 2.5.C, 2.5.D). Shoot P concentrationsof plants supplied with organic material heterogeneously distributed(Ht) were higher than thoseof plants supplied with organic material homogeneously distributed(Hm) (Ht vs Hm; Fig. 2.9 and Tab. 2.5.B). Supply of leaf material (Le) increased shoot P concentrations to a greater extent than supply of stem material (St) (Le vs. St; Fig. 2.9 and Tab. 2.5.B).

Table 2.5.A: A Three-Way ANOVA was performed on data obtained for the treatments that received mineral P supply only. The tested treatments were level of mineral P supply (LP, HP), AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these mains factors is indicated by a star. Significant interactions ($P<0.05$) are also given. In case the ANOVA indicated a significant effect of AM inoculation, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different AM inoculation treatments differ. The results are shown in last row.

Treatments	df	P concentration in the shoot	P concentration in the roots	N concentration in the shoot	N concentration in the roots
Main factors:					
Mineral P supply	1	ns	ns	*	*
AM inoculation	2	*	*	*	*
Bacteria inoculation	1	ns	ns	ns	ns
Interactions:					
Mineral P supply x AM inoculation	2	ns	ns	ns	*
AM inoculation x bacteria inoculation	2	ns	ns	ns	*
DMRT for AM inoculation		MM > MO > NM	MM, MO > NM	NM > MO, NM	NM > MO, MM

Table 2.5.B: A Four-Way ANOVA was performed on data obtained for the treatments that were supplied with organic material. The tested treatments were AM inoculation (MM, MO, NM), bacteria inoculation (+B, -B), type of organic material (Le, St) and distribution of organic material (Hm, Ht). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interactions ($P<0.05$) are also given. For further explanation see Tab. 2.5.A.

Treatments	df	P concentration in the shoot	P concentration in the root	N concentration in the shoot	N concentration in the root
Main factors:					
AM inoculation	2	*	*	*	*
Bacteria inoculation	1	ns	ns	ns	ns
Type of organic material (OM)	1	*	ns	ns	ns
Distribution of organic material (OM)	1	*	*	*	*
Interactions:					
AM inoculation x OM distribution	2	ns	ns	ns	*
Bacteria inoculation x OM distribution	1	ns	ns	ns	*
OM type x OM distribution	1	ns	ns	*	ns
AM inoculation x OM type x OM distribution	2	*	ns	ns	ns
DMRT for AM inoculation		MM > MO > NM	MM, MO > NM	NM > MO > MM	NM > MO, MM

Table 2.5.C: A Three-Way ANOVA was performed on data obtained for the treatments that were supplied with mineral P in high level (HP) and organic material heterogeneously distributed (Ht). The tested treatments were high level of P supply (HP, LeHt, StHt), AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interactions are also given. In case the ANOVA indicated a significant effect of either high level P supply or AM inoculation, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different either high level P supply or AM inoculation treatments differ.

Treatments	df	P concentration in the shoot	P concentration in the root	N concentration in the shoot	N concentration in the root
Main factors:					
High level P supply	2	*	ns	*	*
AM inoculation	2	*	*	*	*
Bacteria inoculation	1	ns	ns	ns	ns
Interactions:					
High level P supply x AM inoculation	4	ns	*	*	*
High level P supply x bacteria inoculation	2	ns	ns	ns	ns
DMRT for high level P supply		HP, LeHt > StHt	-	HP > LeHt > StHt	HP > LeHt, StHt
DMRT for AM inoculation		MM > MO > NM	MM, MO > NM	NM > MO, MM	NM > MO, MM

Table 2.5.D: A Three-Way ANOVA was performed on data obtained for the treatments that were supplied with mineral P in high level (HP) and organic material homogeneously distributed (Hm). The tested treatments were high level of P supply (HP, LeHm, StHm), AM inoculation (MM, MO, NM) and bacteria inoculation (+B, -B). A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interactions are also given. For further explanation see Tab. 2.5.C.

Treatments	df	P concentration in the shoot	P concentration in the root	N concentration in the shoot	N concentration in the root
Main factors:					
High level P supply	2	*	*	*	*
AM inoculation	2	*	*	*	*
Bacteria inoculation	1	ns	ns	ns	ns
Interactions:					
High level P supply x AM inoculation	4	*	*	*	ns
High level P supply x bacteria inoculation	2	ns	ns	ns	ns
DMRT for high level P supply		HP > LeHm, StHm	HP > LeHm > StHm	HP > StHm, LeHm	HP > LeHm, StHm
DMRT for AM inoculation		MM > MO > NM	MM, MO > NM	NM > MO, MM	NM > MM, MO

Application of AM fungi increased shoot P concentrations in all plants, independent of mineral P or organic material supply (Fig. 2.9 and Tabs. 2.5.A, 2.5.B). Plants colonized with AM fungi from minerally fertilized plots (MM) had higher P concentrations in the shoot than plants colonized with AM fungi from organically fertilized field plots (MO) (MM vs. MO; Fig. 2.9 and Tab. 2.5.A, 2.5.B). The effect of bacteria inoculation (+B vs. –B) on P concentrations in the shoot was not significant.

P concentrations in the roots (only roots outside the patches were measured) were not affected by the level of mineral P supply (LP vs. HP; Fig. 2.9 and Tab. 2.5.A). P concentrations in the roots of plants supplied with the higher level of mineral P were not significantly different from P concentrations in the roots of plants supplied with organic material (leaf or stem) heterogeneously distributed (HP vs. Ht; Fig. 2.9 and Tab. 2.5.C) and were significantly higher than root P concentrations of plants supplied with organic material homogeneously distributed (HP vs. Hm; Fig. 2.9 and Tab. 2.5.D). Plants supplied with organic material heterogeneously distributed (Ht) had higher P concentrations in the roots than plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.9 and Tab. 2.5.B).

The type of organic material that was supplied (Le vs. St) had no significant effect on root P concentrations (Fig. 2.9 and Tab. 2.5.B). Inoculation with AM fungi increased root P concentrations in plants supplied either with mineral P or organic material. There was no significant difference between AM fungi from minerally and organically fertilized field plots in the effect on root P concentrations (MM vs. MO; Fig. 2.9 and Tabs. 2.5.A, 2.5.B). Bacteria inoculation (+B vs. –B) had no significant effect on root P concentrations (Fig. 2.9 and Tabs. 2.5.A, 2.5.B).

2.4.9 NITROGEN CONCENTRATIONS IN THE SHOOT AND IN THE ROOT

Shoot N concentrations in plants supplied with the higher level of mineral P (HP) were lower than in plants supplied with the lower level of mineral P (LP) (HP vs. LP; Fig. 2.10 andTab. 2.5.A). Shoot N concentrations in plants supplied with the higher level of mineral P (HP) were significantlyhigher than that in plants supplied with organic material (leaf or stem) either heterogeneously (Ht) or homogeneously distributed (Hm) (HP vs. Ht, Hm; Fig. 2.10 and Tabs. 2.5.C, 2.5.D). Shoot N concentrations in plants supplied with organic material heterogeneously distributed (Ht) were higher than in plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.10 and Tab. 2.5.B). Of

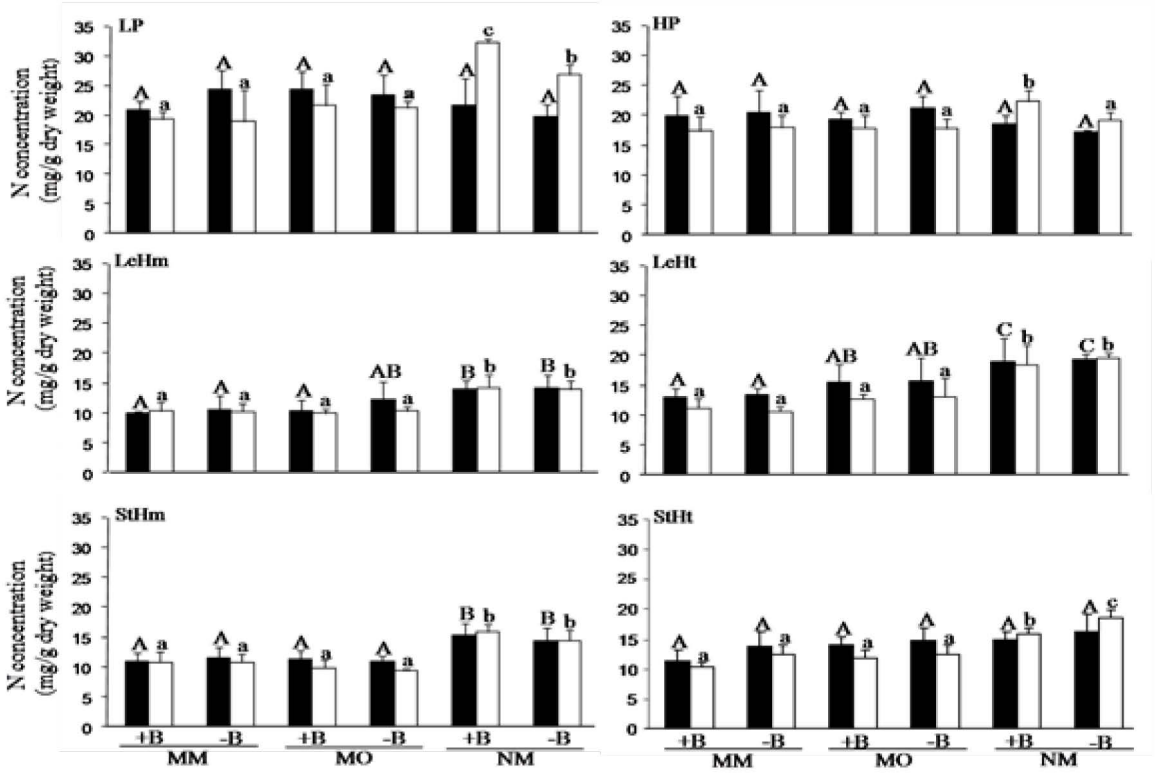

Figure 2.10: N concentrations in the shoot (black bar) and in the root (white bar). For further explanation see Fig. 2.9.

all treatments, plants supplied with the lower amount of mineral P (LP) had the highest N concentrations in the shoot.

The type of organic material (Le vs. St) had no significant effect on N concentrations in the shoot (Fig. 2.10 and Tab. 2.5.B). Inoculation with AM fungi significantly decreased shoot N concentrations only when plants were supplied with organic material (Fig. 2.10 and Tab. 2.5.B). Shoot N concentration was not significantly affected by bacteria inoculation (+B vs. –B; Fig. 2.10 and Tabs. 2.5.A, 2.5.B).

Supply of the higher level of mineral P (HP) compared with supply of the lower level of mineral P (LP) decreased N concentrations in the roots (HP vs. LP; Fig. 2.10 and Tab. 2.5.A). However, plants supplied with the higher level of mineral P had higher root N concentrations than plants supplied with organic material either heterogeneously (Ht) or homogeneously distributed (Hm) (HP vs. Ht, Hm; Fig 1.9 and Tabs. 2.5.C, 2.5.D). Nitrogen concentrations in the roots were higher in plants supplied with organic material heterogeneously distributed (Ht) than in plants supplied with organic material homogeneously distributed (Hm) (Ht vs. Hm; Fig. 2.10 and Tab. 2.5.B). Among all

treatments, root N concentrations were highest in plants supplied with the lower level of mineral P (LP).

The type of organic material (Le vs. St) had no significant effect on root N concentrations (Fig. 2.10 and Tab. 2.5.B). Application of AM fungi significantly decreased N concentrations in the roots (Fig. 2.10 and Tabs. 2.5.A, 2.5.B). There was no significant difference in root N concentrations between AM fungi from minerally (MM) and from organically (MO) fertilized field plots (MM vs. MO; Fig. 2.10 and Tabs. 2.5.A, 2.5.B). Bacteria inoculation had no significant effect on root N concentration (Fig. 2.10 and Tabs. 2.5.A, 2.5.B).

2.5 DISCUSSION

In the present study, the rate of AM root colonization by AM fungi from minerally and organically fertilized field plots was not significantly different when plants were supplied with either mineral P or organic material. Long-term application of mineral and organic fertilizer in the field thus did not have a significant effect on the ability of indigenous AM fungi to form mycorrhiza under the present experimental condition. Sweet potato plants clearly benefited from the AM symbiosis with respect to growth and P uptake. Plant colonized by AM fungi from minerally field plots tended to have increased plant dry weight compared to plants colonized by AM fungi from organically fertilized field plots when plants were supplied with either mineral P or with organic material. Johnson et al. (2010) reported that AM fungi generally perform best in their endemic soil. Thus, it was expected in the present study that AM fungi from minerally fertilized field plots may turn out to be superior in the treatment with mineral P supply; and, vice versa, that AM fungi from organically fertilized field plots may turn out to be superior in the treatments with organic material supply. This expectation was not supported, and AM fungal contribution to plant P uptake and growth was in most cases similarly high, irrespective of origin. This indicates that the AM fungi from the different field plots were able to quickly adapt to the P supply conditions in the present experiment. Mycorrhizal fungal communities may retain their genetic ability for adaptation to new environmental conditions even after living a long time in a soil with a particular set of conditions.

The AM fungi from both minerally and organically fertilized field plots in the present study did not respond positively to the supply of organic material. This was indicated by

decreased AM root colonization rates in organic patches (in Ht treatments) and by decreased total AM root colonization rates when the organic materials were homogeneously distributed (in Hm treatments). The decreased AM root colonization due to supply of organic matter might be caused by an increasing mineral P supply form mineralized organic P during organic matter decomposition. Plant organic matter contains mineral P as well as organic P (Joner and Jakobsen, 1995a). Increasing mineral P can reduce the ability of AM fungi to colonize the roots (Song et al., 2011). The negative effect of organic matter on AM colonization might also be caused by the competitive interactions between microorganisms stimulated by the supplied organic matter and AM fungi (Hodge, 2001).

An increase in mineral P availability in the soil caused by supply of organic matter may also be indicated by theincreased plant growth of non-mycorrhizal plants supplied with organic matter either homogeneously (Hm) or heterogeneously (Ht) distributed compared to plants supplied with low mineral P (LP). Phosphorus is essential element for plant growth and is involved in many plant metabolic functions (Nelson and Janke, 2007). However, total P content of non-mycorrhizal plants supplied with organic material either homogeneously or heterogeneously distributed was not significantly differentfrom non-mycorrhizal plants supplied with low mineral P, and total N content of non-mycorrhizal plants supplied with the low P level was higher than that of non-mycorrhizal plants supplied with organic material homogeneously distributed.

Both shoot dry weight and shoot/root ratio was increased by an addition of mineral P and by inoculation of AM fungi in the treatment with lower mineral P supply. Under low mineral P supply, plants increase transport of photosynthates relatively more to the roots than to the shoot (Marschner et al., 1996; Hammond and White, 2011). With addition of mineral P, more photosynthates are directed to the shoot and are less available to the root (Harris, 1992). The mycorrhizal associationcan increase shoot/root ratios because mycorrhizal plants have a greater ability to absorb nutrients compared to non-mycorrhizal plants (Smith and Read, 1997, p. 236). The absorbing surface area of the root is greatly increased by AM fungal extraradical hyphae (Mukherjee and Ané, 2011; Rakshit and Bhadoria, 2008) and thus mycorrhizal plants can allocate more resources to the shoot than to the roots (Marschner, 1995, p. 572; Vega-Frutis et al., 2011).

Plant P and N uptake and hence plant growth were increased not only by the high level mineral P but also by the supply of organic material. Even though additional mineral N supply to plants treated with leaf organic material was lower than to plants treated with stem material, leaf material tended to be superior in increase of plant growth compared to stem

material. The superiority of leaf material over stem material in providing plants with adequate nutrition may be caused by the faster rate of decomposition (Jian-Hui et al., 1998) and the higher nutrient concentration in leaf material compared to stem material. Also inthe present experiment, leaf material had higher N concentrations than stem material (see Materials and Methods). It is possible that the higher C/N ratio (lower N concentration) in the stem material led to an increased demand of soil bacteria for mineral N and P, and that this decreased the availability of these elements to the plant (Horwarth, 2005).

The ability of roots to utilize P released from organic material is crucial under low P soil conditions. Non-mycorrhizal plants supplied with organic material placed in the patches (Ht) had higher plant P and N uptake than non-mycorrhizal plants supplied with organic material homogeneously distributed (Hm; Figs. 2.7 and 2.8). Roots are very adaptive in modifying growth to concentrate their efforts in areas that are most profitable (Hodge, 2009). Plants in the present experiment responded to soil patches with organic material (Ht treatments) by proliferating roots within these patches, as shown by a higher proportion of root dry weight in the patches related to total root dry weight (Fig. 2.4, Tab. 2.2). Nevertheless, the higher P and N uptake of non-mycorrhizal plants supplied with organic materials placed in the patches (Ht treatment) was not translated into an increased total plant dry weight when compared with total plant dry weight of non-mycorrhizal plants supplied with organic material homogeneously distributed (Hm treatment), but was translated into increased shoot dry weight only. The non-mycorrhizal plants took up more P and produced much more dry weight with high mineral P supply (HP) compared with non-mycorrhizal plants supplied with organic material (Le or St). Soluble mineral P fertilizers release their nutrients faster than most organic fertilizers (Makinde et al., 2007) and can immediately supply the nutrients needed by the plant.

Root proliferation in organic patches as represented by the proportion of root dry weight in the patches related to total root dry weightwas not significantly different in this study between non-mycorrhizal and mycorrhizal plants. Thus, AM fungi did not decrease root proliferation in organic patches. The AM fungi may enhance nutrient capture for the associated host plant from organic patches, but AM fungi might also enhance P uptake from outside the patch. Grace et al. (2009) suggested that AM fungi increase plant nutrient uptake not only via AM extraradical hyhae but also via the root epidermis and root hairs. Hodge (2006) reported that rootsare more responsive than AM hyphae when both are exploring the same organic patches. This was supported by decreased AM root colonization in organic patches in the present study. In contrast, AM root colonization outside organic patches (Ht)

was not significantly different from AM colonization levels in soil with the lower amount of mineral P supply (LP).

Unfortunately, root P and N concentrations of root growing in the patches with organic material could not be measured in the present study because there was not enough root material for nutrient analysis. The inadequate root sample size from the patches for nutrient analysis was due to the fact that half of the collected roots from the patches were used for measurement of AM root colonization in the patches.

Hyphae length inside the patches was not measured because there was no representative comparison possible between hyphae in organic patches (bottles filled with organic material) and in bulk soil with or without organic material. In organic patches, not only hyphae from AM fungi but also from other fungi were present, and a competition for the same resources likely occurred between them. In bulk soil, in contrast, likely almost only AM hyphae were present. However, there are many reports about the positive correlation between the rate of AM colonization and the growth of AM hyphae (Bressan, 2002; Heinemeyer and Fitter, 2004; de Andrade and da Silveira, 2008). In the present study, the rate of AM colonization was decreased by organic material supplied in either patches or in bulk soil.

Bacteria inoculation had no significant effect on plant nutrient uptake, even in plants supplied with organic material. It is possible that the plant-borne acid phosphatase activity in the rhizoplane was higher than that in the rhizosphere or bulk soil that is due to bacterial activity (Marschner, 1995, p.560). Moreover, wind or water easily transport bacteria spores into open pots, so that bacteria may have been present in equal number in all pots.

There was a large difference in plant performance between mycorrhizal and non-mycorrhizal plants when organic material was applied to the patches. In contrast, there was no large contribution of AM fungi to plant dry weight when the higher amount of mineral P fertilizer (HP) was applied. For use of organic material as organic fertilizer, C/N ratio and N concentration of the organic material must be considered because the decomposition of organic material with high C/N ratio may result in a net loss of plant available N in soil through the process of immobilization.

In conclusion, the present experiment showed that

a) AM fungal colonization distinctly increased growth of sweet potato plants, and this was related to increased P content of mycorrhizal plants.
b) There was no indication that mycorrhizal fungi from a field with long-term history of organic fertilization were superior in the exploitation of organic material in soil

compared with mycorrhizal fungi from a field with long-term history of mineral fertilization.

c) Inoculation with soil bacteria did not significantly effect plant growth or plant P or N content.
d) Application of organic material (finely ground plant material from maize leaf or stem) in the patches to soil caused in the patches an increased rooting density, but not increased root AM colonization rates, and
e) The uptake of P and in particular of N from organic material was higher when the material was applied in patches rather than homogeneously distributed in the total soil volume.

In summary, this study created new evidence that plants can benefit from and exploit patches of organic material in soil. Patches of organic material may be an important source of nutrients for plants, as has previously been shown for patches of mineral nutrients. This study confirmed much previous evidence in the important contribution of AM fungi to plant P uptake. It tested also the contribution of AM fungi in plant nutrient uptake from soil patches of organic material. There was little evidence to indicate that AM fungi have specific abilities that further increase the plants ability of patch utilization. Rather, AM fungi appear to be efficient in P uptake from large soil volume with relatively low amounts of mineral P.

3. THE RESPONSE OF MYCORRHIZAL AND NONMYCORRHIZAL SWEET POTATO ROOT SYSTEMS TO HOMOGENEOUS AND HETEROGENEOUS PHOSPHORUS AND NITROGEN SUPPLY IN SOIL

3.1 ABSTRACT

The distribution of nutrients in the soil is never uniform. Root systems may respond to nutrient heterogeneity in the soil by root proliferation in the nutrient-rich soil zones. Root response to nutrient heterogeneity may be influenced by colonization with arbuscular mycorrhizal (AM) fungi. Therefore, in the present experiment the response of non-mycorrhizal and mycorrhizal plants to nutrient heterogeneity in soil was studied using sweet potato (*Ipomea batatas* L.). Plants were grown in split-root pots with different rates of either P or N supply to the halves of the root system of single plants. The total amount of either P or N over two root halves of each split-root pot was the same. Plants were either left uninoculated or were inoculated with an isolate of *G. intraradices*. Shoot dry weight was drastically increased by mycorrhizal colonization, but was not significantly affected by heterogeneous P or N distribution in soil. Belowgroundresponses to soil nutrient heterogeneity, particularly to P supply heterogeneity, were modified by AM fungal colonization. Tuber formation was increased in response to local P supply in non-mycorrhizal, but not in mycorrhizal plants. Extraradical mycelium length and dry weight of *G. intraradices* was not increased in soil patches rich in P or N, indicating that *G. intraradices* may not actively forage for patches with mineral P or N in soil. The present experiment shows that plant response to soil nutrient heterogeneity, and in particular to soil P heterogeneity, can be affected by mycorrhizal colonization, so that conclusions from earlier model studies on heterogeneity effects carried out with non-mycorrhizal plants may not be valid for plants growing in agricultural or natural soils.

3.2 INTRODUCTION

The distribution of resources in soil is never uniform (Weerasinghe and Tanner, 2006). It is widely believed that plant root systems respond to heterogeneity of soil resources by proliferation in the most nutrient rich zones or patches (Peterson et al., 2006). However, root systems can use two different main plasticity mechanisms to respond to the non-uniformity of their environment: (1) increasing the rate of nutrient uptake in the local zones with high nutrient supply (physiological plasticity; rapid and reversible), (2) increasing root proliferation in that zone (morphological plasticity; slow and irreversible) (Ma and Rengel, 2008). Increased physiological plasticity may be especially important for mobile forms of nutrients like nitrate, while morphological plasticity may be more important for exploiting

immobile nutrients such as P (Caldwell et al., 1991). However, root proliferation in inorganic N rich patches is common (Gregory, 2006,p. 158; Shen et al., 2011). The degree of root proliferation in the nutrient rich patches appears to be modulated by the nutrient concentration in the patch, the nutrient demand of plant, the type of nutrient (Caldwell, 1994; Fransen et al., 1999), and the size of patches (Hutchings and Wijesinghe, 1997). By local root proliferation in the nutrient rich patch, roots can absorb more nutrients than roots growing in the nutrient poor soil zone (George et al., 1997). In some observations, plants grown in soil with nutrient added in patches produced more above- and belowground biomass compared with plants supplied with a homogenous nutrient distribution (Lamb et al., 2004).

In sweet potato plants, the root system is divided into fibrous roots which absorb nutrients and water and storage roots which store photosynthetic products (Huaman, 1992; Eguchi, 2000). The contribution of N to storage root (tuber) formation is variable (Belehu, 2003). The application of N in the soil can increase tuber formation (Roy et al., 2006, p.253), while Magagula et al. (2010) reported that a high amount of N in soil delays tuber formation in sweet potato plant. Many studies showed that P application to the soil does not have a significant effect on tuber yield (Abdissa et al., 2012; FAO, 2005, p. 26). However, P deficiency in sweet potato limits tuber production (Taraken et el., 2010). Farzana et al. (2009) reported that tuber formation correlated positively with nutrient uptake.

Most plant species form mycorrhizal associations (Smith and Read, 1997, p. 11). This association helps plants to acquire nutrients, particularly P. The AM fungi may also influence root morphological plasticity to forage nutrients in a patch (Wijesinghe et al., 2001). The extraradical hyphae of AM fungi can also proliferate in both organic and inorganic nutrient rich patches (Smith and Read, 2008, p. 158), so that the mycorrhizal association may reduce the requirement of the root system to proliferate in the patch (Farley and Fitter, 1999; Fitter et al., 2000; Tibbett, 2000). On the other hand, a common effect of mycorrhizal colonization on the overall root system is increased root branching (Berta et al., 1995; Gutjahr et al., 2009). The AM fungus, through its external hyphal network, is not only contributing to the uptake of mineral ions by its host but is also representing a large carbon sink within the soil (Miller and Jastrow, 2000). A number of studies showed that the increased rate of ion uptake is sometimes associated with a higher sugar concentration in mycorrhizal roots because of the requirement of energy in active transport (Chesworth, 2008, p. 568; Lejay et al., 2008). The carbon sink strength of roots can be positively correlated with AM fungal colonization levels. In consequence, low AM colonization results in less or no increase in C transfer to the mycorrhizal root (Lerat et al., 2003; Olsson et al., 2002).

Soil environmental conditions as well as plant nutrient status may affect the development and the formation of arbuscular mycorrhizal fungi (Liu et al., 2004). High P concentrations in soil reduce the level of AM fungal root colonization (Gabriel-Neumann et al., 2011), the production of mycorrhizal spores (Lakshmipathy et al., 2012), and the growth of external hyphae (Olsson et al., 2002). In contrast, N supply to soil can decrease, increase, or may have no effect on AM colonization of roots, depending on the P concentration in soil. The AM colonization may be increased with N supply when P is limited, but may be decreased when P is not limited. Furthermore, the extraradical structure (hyphae and spores) are more responsive to N supply than are intraradical structures (Johnson et al., 2003).

The plant host regulates the AM fungal development (Scervino et al., 2005). Higher P concentrations in the plant tissue, particularly in the root, reduce the level of root colonization (Öpik et al., 2008), production of spores, and formation of secondary external hyphae (Grant et al., 2005). Schreiner and Linderman (2005) also reported that leaf N concentration can be negatively correlated with the level of root AM colonization. For the AM fungal mycelium, it has been shown that the proportion of coarse (runner) hyphae to thin (absorbing) hyphae (Olsson et al., 2006) is also affected by plant P status (Nagy et al., 2009).

The present experiment attempted (1) to determine whether belowground growth is directly modified by the concentration of P or N in soil to which the root is exposed, or whether the effects are determined primarily by the overall P or N status of the shoot, and (2) to determine whether mycorrhizal roots have the same response as non-mycorrhizal roots when exposed to different P or N availability in soil. The two hypotheses considered are: (i) plant belowground response to soil P or N supply is of stronger magnitude than the corresponding response of AM fungi to heterogeneous soil P or N supply (see also Chapter 2 of this thesis); (ii) roots colonized by AM fungi decrease the magnitude of the belowground plant growth response to differences in soil P or N ability.

3.3 MATERIALS AND METHODS

3.3.1 EXPERIMENTAL PLANT PREPARATION

Sweet potato (*Ipomea batatas*) motherplants were grown in nutrient solution containing 3.2 mM N (NH_4NO_3), 0.5 mM P (KH_2PO_4), 1.09 mM K (K_2SO_4 and KH_2PO_4), 2.71 mM Ca ($CaSO_4.2H_2O$), 2.71 mM S (K_2SO_4 and $CaSO_4.2H_2O$), 0.06 mM Fe (Fe-EDTA), 0.02 mM B (H_3BO_3), 4 µM Mn ($MnSO_4.H_2O$), 1.18 µM Zn ($ZnSO_4.7H_2O$), 3.15 µM Cu

($CuSO_4$), and 0.27 µM Mo ($(NH_4)_6Mo_7O_{24}.H_2O$). The nutrient solution was exchanged every three days. Single leaf stem cuttings with three nodes and 10-12 of stem length were obtained from these mother plants and rooted in aerated 2.8 mM $CaSO_4$ solution.

After first roots became visible, the $CaSO_4$ solution was replaced by the same nutrient solution as used for the motherplants, in half strength. Plants were transferred to the experimental pots 15 days after rooting, when the longest roots had a length of approximately 10 cm. The root system was divided into two parts of approximately equal size, before the plants were transferred to split-root pots. In case the root system could not be split into two equal parts, excessive roots were cut off at the stem.

3.3.2 PREPATARION OF THE PLANTING POTS

The young plants with split-root systems were transferred into two compartments of split-root pots. These root compartments (RC) of the split-root pots consisted of two black 1 L plastic pots fastened together, side by side, with adhesive tape. There was no contact of the growth substrate in the two RC of each split-root pot. Sieved (4 mm) C loess soil was heated in a drying oven for 48 hours at 80 °C to eliminate AM fungal propagules. Each RC was filled with 1 kg pasteurized C loess soil at a bulk density of 1.3 g dry soil cm^{-3}. One hyphae compartment (HC) of 55 ml was inserted into each RC. It consisted of a small plastic pot with a latticed wall. The HC was covered by a 30 µm nylon mesh that allowed only hyphae, but not roots to penetrate and enter the HC. The HC contained 120 gram of a mixture of sieved (40 µm) sterilized C loess soil, 1 mm glass beads, and water at the weight ratio of 2:2:1(Neumann and George, 2005). The C loess soil in the RC and in the HC for all treatments was fertilized with 200 mg K (K_2SO_4 and KH_2PO_4), 100 mg Mg ($MgSO_4.7H_2O$, 10 mg Fe (Fe-EDTA), 10 mg Zn ($ZnSO_4.7H_2O$) and 10 mg Cu ($CuSO_4.5H_2O$) kg^{-1} dry soil.

3.3.3 SET-UP OF THE INOCULATION AND FERTILIZATION TREATMENTS

For the mycorrhizal treatments (+M), each RC of the split-roots pots was inoculated with 60 gram inoculum of *Glomus intraradices*. Inoculum was obtained from pot cultures of this fungus with maize cultivated on the same C loess soil as used in the experiment, and consisting of air-dried soil with extraradical AM mycelium, AM spores, and colonized root fragments. The inoculum was mixed homogeneously with the soil before it was filled into the two RC of the split-root pots. For non-mycorrhizal treatments (-M), each RC was inoculated with 60 gram sterilized mycorrhizal inoculum and 40 ml of aqueous filtrate of inoculum (filtered through VWR international no. 313 paper) to encourage a microflora similar to that

in the mycorrhizal treatments. Thereafter, the water content of the soil from both +M and –M were adjusted to approximately 16% w/w by addition of distillated water. The inoculum for -M treatments was sterilized by heating in the oven at 100 °C overnight. The HCs were not inoculated with fungal inoculum. All plants were supplied with additional 100 mg P and 300 mg N in total. The way by which these total amounts of N and P were distributed over the two compartments of each split-root pot differed depending on the treatment. In treatments with homogeneous nutrient supply, both adjacent compartments were supplied with 50 mg P kg^{-1} dry soil (DS) (50:50) and 150 mg N kg^{-1} DS. In the P gradient treatments,

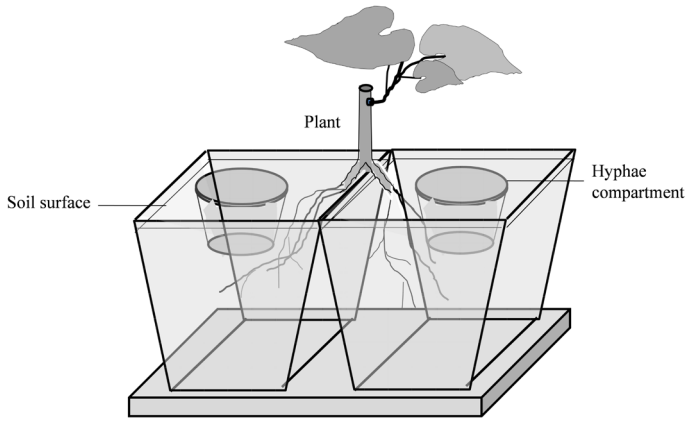

Figure 3.1: The position of plant and hyphae compartments (HC) in the experimental split-root pot.

the P supply level in split-root pots was either (mg kg^{-1} DS) 70:30 or 85:15. In these treatments, the N supply level was 150:150. In the N gradient treatments, the P supply level was 50:50, while the amounts of N were (mg kg^{-1} DS) 180:120, 210:90, or 255:45. The fertilization of the soil in the HCs corresponded to that of respective RCs. The position of the HCs in the experimental split-root pot can be seen in Fig. 3.1.

3.3.4 PLANT GROWTH CONDITIONS

The pots were set up completely randomized in a greenhouse in Grossbeeren (long. 13°20'E; lat. 51°22'N), Germany, for nine weeks from 21 December 2008 to 26 February 2009 with a light period of approximately 8 h day/16 h night. Average light intensity was 700 µmol m^{-2} s^{-1} and there was no addition of artificial light. Average air temperatures in the glasshouse during this time were 23 °C day/20 °C night and relative humidity averaged 70%. All planting pots of this experiment changed their position on the planting table at regular

intervals, but a completely randomized design was maintained. The gravimetric water content of the soil was adjusted to approximately 16% w/w after the plants were inserted. Water loss from the pots was estimated gravimetrically, and was replaced by deionized water every two days. Irrigation water was distributed over the two RCs of each split-root pot according to visual appraisal.

3.3.5 HARVEST AND ANALYSIS OF PLANT AND ARBUSCULAR MYCORRHIZAL FUNGAL MATERIAL

At the time of harvest, shoots were cut off, and the roots (without tubers) and tubers were washed from the soil of each RC with tap water. Roots and tubers were separated by hand. Shoots, and for each RC root and tuber dry weight were measured with a balance after drying at 80 °C for 48 h in the oven. Total plant dry weight (DW) was determined by addition of shoot, root, and tuber DW of each plant. Root or tuber DW in the two RC was determined by root or tuber DW of each plant. Shoot/root ratio was determined by shoot DW divided by total root DW in the two RCs of each plant, while aboveground/belowground ratio was determined by shoot DW divided by root and tuber DW of each plant.

The ratio of the root or the belowground biomass DW of the two halves of the root system in plants supplied with heterogeneous either P or N supply was determined by the root or the belowground biomass DW in the RC that received the higher amount of either P or N divided by the root DW in the RC that received the lower amount of either P or N, respectively. In the cases where plants were supplied with homogeneous either P or N supply, the ratio of the dry weight of the two halves of the root system was determined by the root DW in the RC which received the higher amount of either P or N divided by the root DW in the RC which received the lower amount of either P or N.

In treatments with P or N distributed heterogeneously, of course root or tuber DW of one half of the root system was determined by root or tuber DW in the RC that received either the lower amount of P or N or the higher amount of P or N. In treatments with P or N homogeneously distributed, root or tuber DW in half of the root system was estimated as the total root or tuber DW in both RC divided by two.

The mode of determination of the ratio of the root DW of the two halves of the root system was also used in the determination of the ratio of AM fungal development (the ratios of AM root colonization, hyphae length, ratio coarse to thin hyphae, weight of mycelium, number of spores per mg mycelium and number of spores per m hyphae length) of the two sides (RC+HC) of the split pots exposed to different either P or N treatments. The

determination of root or tuber DW in each half of the root system was also used in the determination of AM fungal development and P and N concentrations in the belowground biomass (root and tuber) in that half of the root system.

To assess the AM fungal colonized root length, representative samples of fresh roots (approximately 1 g) were taken from each of the two root parts of each plant. The root samples were cleared and stained with trypan blue in lactic acid according to Philips and Hayman (1970). Approximately 200 root intersections were counted according to Giovannetti and Mosse (1980). The extent of AM fungal root colonization was expressed as the AM fungal root length in percent of the total root length.

Mycelium was collected from the HC by washing their contents over a 40 µm sieve plate. Collected mycelium was kept in a freezer at -20 °C in plastic tubes filled with water and alcohol (15%) and then it was freeze dried. After the total dry weight of the mycelium had been assessed, subsamples of approximately 500 µg were stained overnight with a few drops of trypan blue in lactic acid. Thereafter, stained mycelium was mixed and fractioned in the blender in 250 ml water. The blender was switched on at level 'low' for 10 seconds, put off for 5 seconds, and switched on again for 20 seconds. Mixed and fractioned mycelium was transferred into 300 ml glass beakers, and then it was stirred with a stirrer on high level. A 90 ml subsample was taken out from the glass beaker with a pipette (the stirrer was left on at level low). During this subsampling, the tip of the pipette was maintained in the same position (2 cm depth in the center of the beaker). The hyphae length and number of spores were assessed by a modified membrane filtration method. The subsample was transferred immediately after sampling into a vacuum pump apparatus. All hyphae were filtered by the vacuum apparatus on a nitrocellulose membrane with gridlines. The nitrocellulose membrane was cut into halves and transferred on slides marked A and B. The amount of grid squares (for examples 32 squares) on the nitrocellulose on slide A and B was determined. After that, the amounts of spores and of hyphae crossings with the grid lines were counted under a light microscope with 200x and 50x magnification, respectively. Thin (ø≤5µm) and coarse (ø>5 µm) hyphae were counted separately.

Hyphae length (m) for each hyphal compartment was then calculated as

$$\text{Number of counted cross sections} \times \frac{\pi}{4} \times \text{Length of one square line (cm)} \times \text{Dilution factor} \times \frac{\text{Total length of all squares lines on area (cm)}}{\text{Number of counted squares} \times \text{Length of one square line (cm)}} \times \frac{1}{\text{Dry weight of subsample of mycelium in HC (mg)}} \times 100 \times \text{Total dry weight of mycelium in HC (mg)}$$

Total hyphae length density (m per cm^3 soil) was calculated as

$$\frac{\text{Coarse hyphae length (m)} + \text{Thin hyphae length (m)}}{\text{Volume of hyphae compartment } (cm^3)}$$

To assess nutrient concentrations in plant tissue, dried shoot and belowground biomass (root and tuber) from each plant was ground into fine powder. Shoots were ground in a rotation mill (ZM 100, Retsch) to the size of 0.25 mm and belowground biomass was ground in a Fritsch Pulverisette mill. Approximately 200 mg of ground plant material were digested for 20 min in Teflon vessels in a microwave, together with 5 ml of 60% HNO_3 and 2 ml 30% H_2O_2. The solution was taken up into 25 ml of distilled water, and filtered through blue ribbon filter paper (Rundfilter Macherey Nagel 616/125 mm). Phosphate concentrations in the liquid samples were measured photometrically (EPOS Analyser 5060) after addition of molybdate-vanadate solution (Gericke and Kurmies, 1952). The total P content of shoots and belowground biomass were calculated by multiplying their dry weight with their P concentration.

The quantitative extraction of N from plant material was done by explosive combustion in an oxygen enriched helium atmosphere surrounded by a copper oxide filled pipe at a temperature of 980°C (Elementar Vario EL). The resulting gas mix was submitted to a gas-phase chromatograph where N could be quantified in a thermal conductivity tube. The total N content of shoot and belowground biomass were calculated by multiplying their dry weight with their N concentration.

3.3.6 STATISTICAL ANALYSIS

The experiment had a completely randomized design with four replicates per treatment. Treatment effects were statistically analyzed by SPSS (SPSS 15, SPSS Inc.

Chicago USA). A Two-Way ANOVA was conducted to assess whether the fertilization treatments and the AM fungi inoculum had a significant effect on the mean values of plant growth and nutrient uptake parameters. A One-Way ANOVA was conducted to assess particularly the effect of AM fungal inoculation. In addition, a Duncan Multiple Range Test was conducted to identify significant differences between the mean values. In all tests, differences were considered significant when $P<0.05$.

3.4 RESULTS

3.4.1 PLANT DRY WEIGHT AFTER HARVEST

The total DW of plants either uninoculated or inoculated with *G. intraradices* was not affected by either P or N supply treatments (Tabs. 3.1.A and 3.1.D). Mycorrhizal plants showed a significantly higher plant DW compared with non-mycorrhizal plants in the treatments with P 50:50 distribution (Tabs. 3.1.A and 3.1.D). Shoot dry weight of plants either uninoculated or inoculated with the AM fungus was also not affected by either P or N supply treatments (Tabs. 3.1.A and 3.1.D). Inoculation with the AM fungus increased shoot dry weight particularly in the N supply treatments (Tabs. 3.1.A and 3.1.D).

Total root DW was not significantly affected by either P or N supply treatments or by inoculation with the AM fungus. However, the ratio of the root DW of the two root parts of the split-root system was affected by P supply treatments depending on the inoculation with the AM fungus (Table 3.1.A). The root DW of non-mycorrhizal plants tended to be increased in the RC that received higher P supply; on the contrary, root DW of mycorrhizal plants tended to be increased in the RC that received lower P supply (Tabs. 3.1.B and 3.1.C). The ratio of the root DW of the two parts of the split-root system was also affected by N supply treatments, but was not affected by inoculation with the AM fungus (Tab. 3.1.D). Root DW of non-mycorrhizal and mycorrhizal plants tended to be higher in the RC that received higher N supply (Tabs. 3.1.E and 3.1.F).

The ratio of the belowground DW of the two halves of the root system was not significantly affected by either P or N supply treatment or inoculation with the AM fungus (Tabs. 3.1.A and 3.1.D). However, tuber dry weight of non-mycorrhizal plants tended to be higher in RCs that received higher amounts of P while tuber dry weight of mycorrhizal plants tended to be higher in RCs that received lower amounts of P (Tabs. 3.1.B and 3.1.C). Tuber DW of mycorrhizal and non-mycorrhizal plants tended to be decreased in the RC that

received the higher amount of N (Tabs. 3.1.E and 3.1.F).

Shoot/root ratio and aboveground/belowground ratio were not affected by either P or N supply treatments as main factor (Tabs. 3.1.A and Tabs. 3.1.D). However, mycorrhizal plants showed a significantly higher shoot/root ratio and in tendency a lower aboveground/belowground ratio. This was due to the fact that the AM fungus increased tuber dry weight (Tab. 3.1.A and Tab. 3.1.B). This effect of the AM fungus on the tuber dry weight occurred irrespective of P or N supply ratios.

Table 3.1.A: Total plant dry weight (DW), root and tuber DW, shoot/root ratio, aboveground/belowground ratio as well as ratio of DW of the two halves of the split-root system of plants exposed to different P supply treatments and inoculated (+M) or not (-M) with an AM fungus.A significant (P<0.05) effect of these main factors is indicated by a star. Significant interaction is also given.

P supply ratio to the two halves of the split-pot system (RCs+HCs)		50:50	70:30	85:15	Statistical significances		
					AM fungus	P supply ratio	Inter-action
Total plant DW (g per plant)	-M	6.29a ± 1.25	7.31ab ± 2.57	8.40abc ± 1.63	*	ns	ns
	+M	10.97c ± 1.53	9.26bc ± 1.56	9.14bc ± 1.65			
Shoot DW (g per plant)	-M	2.97 ± 0.81	4.09 ± 1.76	4.54 ± 1.20	ns	ns	ns
	+M	4.95 ± 0.96	4.96 ± 1.28	4.38 ± 0.30			
Root DW in the two RCs (g per plant)	-M	0.92 ± 0.29	1.39 ± 0.64	1.27 ± 0.29	ns	ns	ns
	+M	1.19 ± 0.22	1.32 ± 0.45	1.23 ± 0.05			
Tuber DW in the two RCs (g per plant)	-M	2.40a ± 0.64	1.65a ± 0.95	2.60a ± 1.05	*	ns	ns
	+M	4.84b ± 0.98	2.99a ± 1.73	3.53ab ± 1.38			
Shoot/root ratio	-M	3.26ab ± 0.17	2.98a ± 0.23	3.57bc ± 0.53	*	ns	*
	+M	4.17d ± 0.35	3.85cd ± 0.43	3.56bc ± 0.16			
Aboveground/ belowground ratio	-M	0.91 ± 0.20	1.40 ± 0.42	1.22 ± 0.41	ns	ns	ns
	+M	0.83 ± 0.19	1.30 ± 0.74	0.99 ± 0.33			
Ratio of root DW of the two halves of the root system	-M	0.85a ± 0.14	1.22c ± 0.20	1.13bc ± 0.16	*	ns	*
	+M	0.89ab ± 0.14	0.81a ± 0.16	0.92ab ± 0.11			
Ratio of belowground DW of the two halves of the root system	+M	0.26 ± 0.20	3.31 ± 1.02	3.20 ± 3.83	ns	ns	ns
	-M	0.21 ± 0.20	1.26 ± 1.81	0.26 ± 0.27			

Values are means and SD four replicates of each treatment combination. Mean values followed by the same letter within the same parameter are not significantly (P<0.05) different.

Table 3.1.B: Root and tuber DW in the root compartments (RC) of the split-root pot that received the lower amount of P.

P supply (mg kg^{-1})		50	30	15	Statistical significances		
					AM fungus	P supply	Inter-action
Root DW	-M	0.46 ± 0.14	0.65 ± 0.33	0.60 ± 0.17	ns	ns	ns
(g per RC)	+M	0.59 ± 0.11	0.74 ± 0.31	0.64 ± 0.02			
Tuber DW	-M	1.20ab ± 0.32	0.38a ± 0.44	0.89ab ± 1.01	*	ns	ns
(g per RC)	+M	2.42bc ± 0.49	1.84abc ± 1.74	3.40c ± 1.65			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly (P<0.05) different.

Table 3.1.C: Root and tuber DW in the RC of the split-root pot that received the higher amount of P.

P supply (mg kg^{-1})		50	70	85	Statistical significances		
					AM fungus	P supply	Inter-action
Root DW	-M	0.46 ± 0.14	0.75 ± 0.31	0.67 ± 0.12	ns	ns	ns
(g per RC)	+M	0.59 ± 0.11	0.57 ± 0.15	0.59 ± 0.06			
Tuber DW	-M	1.20 ± 0.32	1.27 ± 0.75	1.71 ± 1.65	ns	ns	ns
(g per RC)	+M	2.42 ± 0.49	1.15 ± 1.52	0.14 ± 0.28			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly (P<0.05) different.

Table 3.1.D: Total plant DW, root and tuber DW, shoot/root ratio, aboveground/belowground ratio as well as ratio of the two root systems of plants exposed to different N supply treatments and inoculated (+M) or not (-M) with an AM fungus.A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction is also given. In case the ANOVA indicated a significant effect of the N supply ratio, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different N supply ratio treatments differ.

N supply ratio to the two halves of the split pot system (RCs+HCs)		150:150	180:120	210:90	255:45	Statistical significances			DMRT for N supply ratio
						AM fungus	N supply ratio	Inter-action	
Total plant DW (g per plant)	-M	6.29a ± 1.25	6.48a ± 0.62	6.25a ± 1.30	6.36a ± 0.50	*	ns	ns	-
	+M	10.97b ± 1.53	11.23b ± 0.74	9.87b ± 1.57	10.76b ± 1.21				
Shoot DW (g per plant)	-M	2.97a ± 0.81	3.80abc ± 0.23	3.51ab ± 0.80	3.33ab ± 0.55	*	ns	ns	-
	+M	4.95cd ± 0.96	4.65bcd ± 0.66	5.34d ± 1.46	5.44d ± 1.14				
Root DW in the two RCs (g per plant)	-M	0.92 ± 0.29	1.18 ± 0.12	1.11 ± 0.37	0.98 ± 0.22	ns	ns	ns	-
	+M	1.19 ± 0.22	1.10 ± 0.24	1.34 ± 0.44	1.29 ± 0.27				
Tuber DW in the two RCs (g per plant)	-M	2.40ab ± 0.64	1.51a ± 0.76	1.63a ± 0.79	2.05ab ± 0.59	*	*	*	150:150> 120:180> 45:255; 90:210
	+M	4.84de ± 0.98	5.48e ± 0.39	3.19bc ± 0.64	4.03cd ± 1.25				
Shoot/Root ratio	-M	3.26a ± 0.17	3.26a ± 0.46	3.24a ± 0.33	3.47a ± 0.54	*	ns	ns	-
	+M	4.17b ± 0.35	4.28b ± 0.38	4.02b ± 0.21	4.23b ± 0.20				
Above-ground /below-ground ratio	-M	0.91abc ± 0.20	1.48d ± 0.39	1.34cd ± 0.37	1.12abcd ± 0.26	*	ns	ns	-
	+M	0.83ab ± 0.19	0.71a ± 0.10	1.19bcd ± 0.34	1.06abcd ± 0.32				
Ratio of the DW of the two halves of the root system	-M	0.85a ± 0.14	1.19 ± 0.23	1.07abc ± 0.15	1.25c ± 0.22	ns	*	*ns*	45:255> 90:210; 120:180> 150:150
	+M	0.89a ± 0.14	0.93ab ± 0.07	1.10abc ± 0.19	1.25c ± 0.16				
Ratio of belowground DW of the two halves of the root system	-M	0.26 ± 0.20	1.97 ± 1.59	2.40 ± 2.88	0.61 ± 0.64	ns	ns	ns	-
	+M	0.21 ± 0.20	1.25 ± 1.78	0.62 ± 0.54	0.77 ± 0.92				

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 3.1.E: Root and tuber DW in the RC of the split-root pot that received the lower amount of N.

N supply (mg kg^{-1})		150	120	90	45	Statistical significances		
						AM fungus	N supply	Inter-action
Root DW (g per RC)	-M	0.46a ± 0.14	0.54a ± 0.08	0.53a ± 0.16	0.43a ± 0.08	*	ns	ns
	+M	0.59a ± 0.11	0.57a ± 0.13	0.66a ± 0.27	0.57a ± 0.08			
Tuber DW (g per RC)	-M	1.20ab ± 0.32	0.62a ± 0.78	0.56a ± 0.43	1.70abc ± 0.93	*	ns	ns
	+M	2.42bcd ± 0.49	3.51d ± 1.92	3.30bcd ± 0.93	2.75cd ± 0.80			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly (P<0.05) different.

Table 3.1.F: Root and tuber DW in the RC of the split-root pot that received the higher amount of N.

N supply (mg kg^{-1})		150	180	210	255	Statistical significances		
						AM fungus	N supply	Inter-action
Root DW (g per RC)	-M	0.46 ± 0.14	0.63 ± 0.09	0.58 ± 0.22	0.55 ± 0.16	ns	ns	ns
	+M	0.59 ± 0.11	0.53 ± 0.11	0.69 ± 0.17	0.72 ± 0.19			
Tuber DW (g per RC)	-M	1.20 ± 0.32	0.89 ± 0.79	1.07 ± 1.20	0.35 ± 0.42	ns	ns	ns
	+M	2.42 ± 0.49	1.97 ± 1.91	0.89 ± 1.04	1.28 ± 1.97			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly (P<0.05) different.

3.4.2 ARBUSCULAR MYCORRHIZA FUNGAL COLONIZED ROOT LENGTH, HYPHAE LENGTH, RATIO OF COARSE TO THIN HYPHAE, NUMBER OF SPORES, AND AMOUNT OF MYCELIUM OBTAINED FROM THE FUNGAL COMPARTMENTS

In non-inoculated plants, the roots were not colonized by an AM fungus. In inoculated plants, the rates of AM root colonization were 72-83%. The ratios of AM root colonization, hyphae length, coarse to thin hyphae ratio, weight of mycelium, number of spores per milligram mycelium as well as of the number of spores per meter hyphae length of the two parts of the split-root system were not significantly affected by P supply treatments (Tab. 3.2.A). However, the weight of the mycelium was increased in the HCs that received a low P supply (Tab. 3.2.B).

From all the AM fungal parameters measurement, only the ratio of coarse to thin hyphae and the number of spores per milligram mycelium were affected by N supply treatments (Tab. 3.2.D). The ratio of coarse to thin hyphae tended to be higher in HC that received a high N supply, while the number of spores per milligram mycelium tended to be decreased in HC that received higher N supply.

Table 3.2.A: Ratios of AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spores per mg mycelium, and of number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots exposed to different P supply treatments.

P supply ratio in the two sides (RC+HC) of the split-root pots	50:50	70:30	85:15	Signifi-cance
AM colonization	1.00 ± 0.05	0.93 ± 0.08	0.96 ± 0.06	ns
Hyphae length (m/cm^3 soil)	0.95 ± 0.33	0.72 ± 0.25	0.86 ± 0.23	ns
Ratio coarse to thin hyphae	0.87 ± 0.12	1.07 ± 0.17	1.27 ± 0.33	ns
Weight of mycelium (mg per HC)	0.76 ± 0.23	0.79 ± 0.42	0.67 ± 0.02	ns
Number of spores per mg mycelium	1.04 ± 0.12	1.08 ± 0.15	1.07 ± 0.14	ns
Number of spores per m hyphae length	0.83 ± 0.16	1.10 ± 0.12	0.88 ± 0.28	ns

Table 3.2.B: The AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spores per mg mycelium, and number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots that received the lower amount of P.

P supply (mg kg^{-1})	50	30	15	Signifi-cance
AM colonization (%)	82.08 ± 3.79	83.71± 8.89	79.26 ± 6.49	ns
Hyphae length (m/cm^3 soil)	8.93 ± 3.28	16.50 ± 5.70	19.61 ± 7.66	ns
Ratio coarse to thin hyphae	0.39 ± 0.09	0.38 ± 0.16	0.32 ± 0.05	ns
Weight of mycelium (mg per HC)	17.03a ± 6.20	28.24ab ± 13.36	43.80bc ± 10.97	*
Number of spores per mg mycelium	3947 ± 507	3493 ± 418	3312 ± 349	ns
Number of spores per m hyphae length	309 ± 94	239 ± 26	353 ± 170	ns

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 3.2.C: The AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spores per mg mycelium, and number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots that received the higher amount of P.

P supply (mg kg^{-1})	50	70	85	Signifi-cance
AM colonization (%)	82.08 ± 3.79	73.32 ± 6.67	72.74 ± 5.55	ns
Hyphae length (m/cm^3 soil)	8.93 ± 3.28	8.94 ± 4.98	13.36 ± 3.29	ns
Ratio coarse to thin hyphae	0.39 ± 0.09	0.40 ± 0.07	0.50 ± 0.19	ns
Weight of mycelium (mg per HC)	17.03 ± 6.20	17.03 ± 12.88	19.84 ± 4.51	ns
Number of spores per mg mycelium	3947 ± 507	3981 ± 633	3749 ± 775	ns
Number of spores per m hyphae length	309 ± 94	286 ± 40	249 ± 129	ns

Table 3.2.D: Ratios of AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spores per mg mycelium, and of number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots exposed to different N supply treatments.

N supply ratio in the two sides (RC+HC) of the split-root pots	150:150	180:120	210:90	255:45	Signifi-cance
AM colonization	1.00 ± 0.05	1.01 ± 0.03	1.01 ± 0.06	1.05 ± 0.04	ns
Hyphae length (m/cm^3 soil)	0.95 ± 0.33	0.74 ± 0.06	0.74 ± 0.15	0.71 ± 0.45	ns
Ratio coarse to thin hyphae	0.87a ± 0.12	1.18b ± 0.16	1.02ab ± 0.12	1.14b ± 0.18	*
Weight of mycelium (mg per HC)	0.76 ± 0.23	0.74 ± 0.06	0.76 ± 0.14	0.70 ± 0.22	ns
Number of spores per mg mycelium	1.04b ± 0.12	0.86a ± 0.10	0.87a ± 0.03	0.97ab ± 0.09	*
Number of spores per m hyphae length	0.83 ± 0.16	0.87 ± 0.18	0.91 ± 0.15	1.08 ± 0.36	ns

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 3.2.E: The AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spores per mg mycelium, and number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots that received the lower amount of N.

N supply (mg kg^{-1})	150	120	90	45	Signifi-cance
AM colonization (%)	82.08 ± 3.79	79.30 ± 3.44	77.87 ± 6.46	73.72 ± 2.75	ns
Hyphae length (m/cm^3 soil)	8.93 ± 3.28	18.40 ± 7.28	18.23 ± 7.08	22.57 ± 6.78	ns
Ratio coarse to thin hyphae	0.39 ± 0.09	0.32 ± 0.08	0.38 ± 0.08	0.36 ± 0.12	ns
Weight of mycelium (mg per HC)	17.03 ± 6.20	26.90 ± 9.21	22.53 ± 13.15	28.31 ± 4.13	ns
Number of spores per mg mycelium	3947 ± 507	4474 ± 1090	4716 ± 307	4002 ± 549	ns
Number of spores per m hyphae length	309 ± 95	272 ± 43	235 ± 65	219 ± 58	ns

Table 3.2.F: The AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spores per mg mycelium, and number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots that received the higher amount of N.

N supply (mg kg^{-1})	150	180	210	255	Signifi-cance
AM colonization (%)	82.08 ± 3.79	80.99 ± 3.23	79.74 ± 7.18	81.33 ± 4.00	ns
Hyphae length (m/cm^3 soil)	8.93 ± 3.28	10.32 ± 4.60	9.36 ± 2.67	13.20 ± 13.09	ns
Ratio coarse to thin hyphae	0.39 ± 0.09	0.43 ± 0.04	0.40 ± 0.14	0.45 ± 0.05	ns
Weight of mycelium (mg per HC)	17.03 ± 6.20	15.17 ± 6.94	11.53 ± 2.62	14.97 ± 9.96	ns
Number of spores per mg mycelium	3948 ± 507	3366 ± 1167	3564 ± 175	3764 ± 691	ns
Number of spores per m hyphae length	309 ± 95	215 ± 98	194 ± 77	253 ± 136	ns

3.4.3 PHOSPHORUS AND NITROGEN CONCENTRATIONS IN THE PLANT AND TOTAL PLANT PHOSPHORUS AND NITROGEN CONTENT

The total plant P and N content and shoot P and N concentrations of non-mycorrhizal or mycorrhizal plants were not significantly affected by either P or N supply treatments (Tabs. 3.3.A and 3.3.D). Mycorrhizal plants showed drastically increased P and N content and shoot P concentrations at all P or N supply ratios (Tab. 3.3.A and 3.3.D). Shoot N concentrations were also increased in mycorrhizal plants compared to non-mycorrhizal plants, but to a much lesser than for P (Tabs. 3.3.A and 3.3.D).

The belowground biomass (root and tuber) P and N content and P concentration of non-mycorrhizal or mycorrhizal plants were also not affected by either P or N supply

treatments (Tabs. 3.3.B, 3.3.C, 3.3.E, 3.3.F.). However, belowground biomass N concentration was affected by N supply treatments (Tab. 3.3.F). Belowground biomass N concentration tended to be increased in RC that received a high N supply.

Mycorrhizal plants showed significantly increased P content and P concentration in the belowground biomass at all P or N supply ratios (Tabs. 3.3.B, 3.3.C, 3.3.E, 3.3.F). In contrast, N concentrations in the belowground biomass of mycorrhizal plants were significantly lower than in non-mycorrhizal plants in all RC that received different P or N supply (Tabs. 3.3.B, 3.3.E, 3.3.F) except on sides with high P supply (Tab. 3.3.C). However, mycorrhizal plants tended to have higher N content in the belowground biomass compared to non-mycorrhizal plants (Tab. 3.3.E).

Table 3.3.A: Total P content, P concentration in the shoot, total N content, and N concentration in the shoot of plants exposed to different P supply treatments and inoculated (+M) or not (-M) with an AM fungus.

P supply ratio to the two halves of the split-pot system (RCs+HCs)		50:50	70:30	85:15	Statistical significances		
					AM fungus	P supply ratio	Inter-action
Total P content (mg per plant)	-M	4.47a ± 1.84	5.26a ± 1.90	6.77a ± 2.56	*	ns	*
	+M	23.19c ± 3.30	19.92bc ± 3.05	17.63b ± 3.35			
P concentration the shoot (mg g^{-1} DW)	-M	0.70a ± 0.16	0.73a ± 0.05	0.70a ± 0.22	*	ns	ns
	+M	1.75b ± 0.19	3.15c ± 0.19	1.95bc ± 0.10			
Total N content (mg per plant)	-M	113.61a ± 25.53	126.57ab ± 18.07	140.74ab ± 23.18	*	ns	ns
	+M	176.77c ± 17.39	160.63bc ± 15.63	151.32bc ± 18.09			
N concentration in the shoot (mg g^{-1} DW)	-M	20.20ab ± 2.61	18.83ab ± 2.74	17.33a ± 1.60	*	ns	ns
	+M	21.73b ± 3.31	21.95b ± 2.54	21.70b ± 1.15			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 3.3.B: Total P content, P concentration, total N content, and N concentration in the belowground biomass in the RC of the split-root pot that received the lower amount of P.

P supply (mg kg^{-1})		50	30	15	Statistical significances		
					AM fungus	P supply	Inter-action
P content in the root and tuber (mg per RC)	-M	1.15a ± 0.39	0.81a ± 0.56	1.38a ± 1.25	*	ns	ns
	+M	6.73b ± 0.86	5.80b ± 3.35	7.29b ± 3.29			
P concentration in the root and tuber (mg g^{-1} DW)	-M	0.73a ± 0.05	0.75a ± 0.21	0.90a ± 0.29	*	ns	*
	+M	2.40c ± 0.23	3.25bc ± 0.25	1.85b ± 0.44			
N content in the root and tuber (mg per RC)	-M	27.30 ± 5.42	27.25 ± 5.13	30.01 ± 16.43	ns	ns	ns
	+M	35.45 ± 3.97	33.37 ± 13.64	43.64 ± 16.67			
N concentration in the root and tuber (mg g^{-1} DW)	-M	20.23bc ± 1.80	20.05bc ± 0.35	23.13c ± 5.87	*	ns	ns
	+M	14.80ab ± 3.21	13.78a ± 2.81	11.20a ± 1.93			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly (P<0.05) different.

Table 3.3.C: Total P content, P concentration, total N content, and N concentration in the belowground biomass in the RC of the split-root pot that received the higher amount of P.

P supply (mg kg^{-1})		50	70	85	Statistical significances		
					AM fungus	P supply	Inter-action
P content in the root and tuber (mg per RC)	-M	1.15a ± 0.39	1.52a ± 0.53	3.12ab ± 1.61	*	ns	*
	+M	6.73c ± 0.86	4.73bc ± 4.58	1.79b ± 0.65			
P concentration in the root and tuber (mg g^{-1} DW)	-M	0.73a ± 0.05	0.78a ± 0.15	0.93a ± 0.30	*	ns	ns
	+M	2.40b ± 0.23	3.37b ± 0.23	2.45b ± 0.17			
N content in the root and tuber (mg per RC)	-M	27.30 ± 5.42	33.37 ± 13.59	32.45 ± 16.72	ns	ns	ns
	+M	35.45 ± 3.97	21.84 ± 14.84	12.88 ± 2.74			
N concentration in the root and tuber (mg g^{-1} DW)	-M	20.23 ± 1.83	17.03 ± 3.13	15.03 ± 3.09	ns	ns	*
	+M	14.80 ± 3.21	15.35 ± 4.86	18.20 ± 1.96			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly (P<0.05) different.

Table 3.3.D: Total P content, P concentration in the shoot, total N content, and N concentration in the shoot of plants exposed to different N supply treatments and inoculated (+M) or not (-M) with an AM fungus.

N supply ratio to the two halves of the split-pot system (RCs+HCs)		150:150	180:120	210:90	255:45	Statistical significances		
						AM fungus	N supply ratio	Inter-action
Total P content (mg per plant)	-M	4.47a ± 1.84	4.40a ± 0.45	5.34a ± 1.37	4.78a ± 0.65	*	ns	ns
	+M	23.19b ± 3.30	20.30b ± 2.55	23.23b ± 3.11	21.42b ± 1.80			
P concentration the shoot (mg g^{-1} DW)	-M	0.70a ± 0.16	0.63a ± 0.10	0.70a ± 0.24	0.75a ± 0.17	*	ns	ns
	+M	1.75b ± 0.19	1.73b ± 0.19	2.05b ± 0.17	1.95b ± 0.37			
Total N content (mg per plant)	-M	113.61a ± 25.53	114.21a ± 6.85	134.64a ± 10.50	117.23a ± 4.95	*	ns	ns
	+M	176.77b ± 17.39	166.95b ± 14.13	184.04b ± 18.64	193.87b ± 23.48			
N concentration in the shoot (mg g^{-1} DW)	-M	20.20abc ± 2.61	17.16a ± 0.95	19.74ab ± 1.07	18.20a ± 1.45	*	ns	ns
	+M	21.73bc ± 3.31	21.51bc ± 1.28	23.12c ± 3.17	23.06c ± 1.52			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 3.3.E: Total P content, P concentration, total N content, and N concentration in the belowground biomass in the RC of the split-root pot that received the lower amount of N.

N supply (mg kg^{-1})		150	120	90	45	Statistical significances		
						AM fungus	N supply	Inter-action
P content in the root and tuber (mg per RC)	-M	1.15a ± 0.39	0.92a ± 0.38	1.12a ± 0.92	1.46a ± 1.17	*	ns	ns
	+M	6.73b ± 0.89	7.41b ± 3.68	6.91b ± 1.82	6.91b ± 1.75			
P concentration in the root and tuber (mg g^{-1} DW)	-M	0.73a ± 0.05	0.90a ± 0.27	0.90a ± 0.52	0.63a ± 0.25	*	ns	ns
	+M	2.40c ± 0.23	1.88b ± 0.30	3.33bc ± 0.28	3.10bc ± 0.34			
N content in the root and tuber (mg per RC)	-M	27.30ab ± 5.42	21.18a ± 8.20	20.21a ± 5.93	33.21ab ± 12.73	*	ns	ns
	+M	35.45ab ± 3.97	39.25b ± 19.58	36.49ab ± 7.33	37.56ab ± 9.55			
N concentration in the root and tuber (mg g^{-1} DW)	-M	20.23c ± 1.80	20.65c ± 5.52	20.18c ± 6.04	15.28b ± 0.99	*	ns	ns
	+M	14.80ab ± 3.21	9.85a ± 1.07	12.43ab ± 0.67	11.33ab ± 0.38			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 3.3.F: Total P content, P concentration, total N content, and N concentration in the belowground biomass in the RC of the split-root pot that received the higher amount of N.

N supply (mg kg^{-1})		150	180	210	255	Statistical significances			DMRT for N supply
						AM fungus	N supply	Inter-action	
P content in the root and tuber (mg per RC)	-M	1.15ab ± 0.39	1.10ab ± 0.47	1.71abc ± 1.25	0.81a ± 0.72	*	ns	ns	-
	+M	6.73d ± 0.86	4.79d ± 2.92	4.42cd ± 2.95	4.04bcd ± 3.02				
P concentration in the root and tuber (mg g^{-1} DW)	-M	0.73a ± 0.05	0.78a ± 0.32	1.00a ± 0.14	0.78a ± 0.32	*	ns	ns	-
	+M	2.40bc ± 0.23	2.08b ± 0.33	2.83c ± 0.46	3.33bc ± 0.59				
N content in the root and tuber (mg per RC)	-M	27.30 ± 5.42	27.96 ± 7.27	41.14 ± 11.72	24.89 ± 11.16	ns	ns	ns	-
	+M	35.45 ± 3.97	28.32 ± 13.77	27.63 ± 13.13	31.71 ± 21.70				
N concentration in the root and tuber (mg g^{-1} DW)	-M	20.23a ± 1.80	20.00a ± 4.71	21.63a ± 5.92	29.83b ± 5.90	*	*	ns	255≥210 ≥180;150
	+M	14.80a ± 3.21	13.60a ± 4.80	20.40a ± 6.41	19.68a ± 6.15				

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

3.5 DISCUSSION

The main benefit of mycorrhizal symbiosis to host plants is that the AM fungus helps the plant in P acquisition from the soil by extraradical fungal hyphae, especially from root-distant soil not depleted of nutrients by the root (George, 2000). It was shown in the present experiment that plant P uptake and P concentration in above-and belowground parts of mycorrhizal plants were increased compared to non-mycorrhizal plants. Phosphorus is essential for plant growth and is involved in many metabolic functions (Nelson et al., 2007). Hence, total plant dry weight of sweet potato plantsexposed different either P or N supply was drastically increased in the present experiment by inoculation with *Glomus intraradices*.Extraradical fungal hyphae spread in the soil also increases surface area of the root system to absorb N and subsequently can increase plant N uptake (Neumann and George, 2009). Irrespective of P or N distribution in the soil, also in the present experiment total plant N content and N concentration in the shoot of mycorrhizal plants tended to be higher than in non-mycorrhizal plants.

On the other hand, N concentration in the belowground biomass of mycorrhizal plants tended to be lower than in the belowground biomass of non-mycorrhizal plants. Tuber dry

weight was distinctly increased in mycorrhizal plants compared to non-mycorrhizal plants. Tuber formation can be correlated positively with plant nutrient uptake (Farzana et al., 2009). The lower N concentration in the belowground biomass of mycorrhizal plants is related to lower N concentrations in the tubers. Sweet potato tubers are rich in carbohydrates (Lebot, 2009, p. 89), and therefore at high rates tuber formation dry matter accumulation belowground increases more rapidly than the rate of nutrient accumulation, resulting in lower final belowground nutrient concentrations (Jarrell and Beverly, 1981). The higher tuber dry weight in mycorrhizal plants caused the aboveground/belowground ratio of mycorrhizal plants to be lower than that of non-mycorrhizal plants, particularly in plants exposed to different N supply ratios.

Shoot/root ratio was also increased in plants inoculated with the AM fungus. Mycorrhizal plants have a greater ability to absorb nutrients compared to non-mycorrhizal plants (Smith and Read, 1997, p. 236) because the absorbing surface area of the root is greatly increased by AM fungal extraradical hyphae (Mukherjee and Ané, 2011; Rakshit and Badhoria, 2008), so that mycorrhizal plants can allocate less resources to the root (Marschner, 1995, p. 572; Vega-Frutis et al., 2011).

The ratio of root dry weight of the two parts of the root system was affected by the AM fungus in plants exposed to different P supply, but not in plants exposed to different N supply. This indicates that non-mycorrhizal and mycorrhizal plants can show a different root response to differences in P supply to different parts of the root system. Root dry weight of non-mycorrhizal plants tended to be higher in RC that were supplied with more P, while root dry weight of mycorrhizal plants tended to be higher in RC that were supplied with less P. In the present study, mycorrhizal colonization tended to be increased in RC that received lower P supply, and this increased AM colonization may have caused increased root biomass in the respective RC. Lerat et al. (2003) suggested that the carbohydrate supply to the root system positively correlated with the development of AM fungi in the roots. Apparently, the mycorrhizal association may reduce root proliferation in soil nutrient patches with a higher amount of P.

Both non-mycorrhizal and mycorrhizal plants showed the same root response to differences in N supply in the two RC. Root dry weight of both non-mycorrhizal and mycorrhizal plants tended to be higher in the RC that were supplied with more N. Roots respond sometimes to inorganic N-rich patches by root proliferation (Gregory, 2006, p. 158). Because inorganic N (in particular NO_3^-) can readily move to the roots via diffusion, it has been assumed that roots would not require mycorrhizal assistance to capture inorganic N

(Hodge and Fitter, 2010). Furthermore, the potential benefit to plants of fungal mediated N uptake is not as large as for P (George et al., 1995). In the present experiment, plant belowground (root and tuber) response to differences in P or N supply ratios to the two parts of the root system were not significantly affected by the AM fungus. This shows that response of absorbing roots particularly to different P supply ratios in soil patches, but not of plant total belowground growth, may be altered by AM fungi.

Total plant biomass and shoot dry weight of sweet potato plants were not significantly affected by local variation of P and N placement in soil. Some plant species may have the capacity to effectively integrate soil P or N resources when those nutrients are heterogeneously distributed in soil. These plants then have an equal biomass production with plants supplied with P or N homogeneously distributed, when the same quantity of nutrients is supplied (Cui and Caldwell, 1998). The present results indicate that sweet potato plant have a high ability for nutrient translocation within the plant and nutrient integration for shoot growth. This assumption is supported by the lack of significant differences in total plant P and N contents and shoot P and N concentrations between plants supplied with homogenously and heterogeneously located either P or N.

The mechanisms of regulating the activity of plant nutrient uptake may depend on the plant nutrient demand rather than on nutrient concentration in the rooting medium (Imsande and Touraine, 1994). Plant P or N status, particularly the shoot P and N concentrations, controls P and N uptake demand and root proliferation in a highly nutrient rich patch (Lima et al., 2010; Ma and Rengel, 2008), so that in the present case total root dry weight of plants supplied with homogeneous and heterogeneous nutrient distribution was also not significantly different.

On the other hand, shoot P and N concentration of sweet potato plants in the present experiment were indicative of deficiency when compared to standard values (Munson, 1998). Plant deficient in a certain nutrient may take up this nutrient from soil when available to them, irrespective of homogeneous and heterogeneous distribution of that nutrient in the soil. Thus, as expected, in the present experiment there was no difference in shoot biomass production between soil with homogeneous and heterogeneous nutrient distribution. Very likely, the precision of root foraging in the nutrient rich patch was reduced because of nutrient depletion in this patch (Kembel and Cahill, 2005).

Many previous studies reported that plants supplied with nutrients heterogeneously distributed in soil have higher plant biomass production than plants supplied with nutrients homogeneously distributed (Kume et al., 2006; Roiloa and Retuerto, 2006). Similar

resultswere also obtained from a study using sweet potato plant supplied with plant material (leaf or stem) heterogeneously distributed in a relatively small patch size (Chapter 2 of this thesis). Hutching and Wijesinghe (1997) suggested that plants can have several fold increased biomass production if the nutrient distribution in soil is not homogeneous, but concentrated in a small hotspot. This effect may be explained by higher fixation of nutrients after homogeneous nutrient distribution in soil compared to lower rates of nutrient fixation in small patches. The minimum size of a nutrient-rich patch to evoke a root response in that patch is unknown (Hodge, 2006). Conversely, Kume et al. (2006) reported that in larger P patches a greater biomass production resulted in maize plants, by increasing root length in P patches even though the P uptake rate per root was not affected.

In the present experiment, tuber formation was very variable between replications, so that standard differences were high and it was difficult to achieve significant results with the four replications used for this study. Total tuber dry weight of mycorrhizal and non-mycorrhizal plants was not significantly affected by the P supply ratio. However, tuber dry weight of non-mycorrhizal plants tended to be higher in RC that received a higher amount of P. The start of tuber formation is an accumulation of photosynthates, consisting predominantly of starch. The tuber biomass production is then affected by the capacity of tubers to accumulate photosynthates (sink capacity) (Belehu, 2003). This capacity is controlled by the P status of the sink (storage) cell (Atwell et al., 2003, p 184). In the present experiment, tuber dry weight tended to be decreased in RC that received higher amounts of N, particularly in mycorrhizal plants. Large amounts of N in soil can delay tuber formation, decrease cambial activity, and increase lignification, thus favouring the production of non-tuber roots (Magagula et al., 2010).

In the present study the extent of AM colonization did not appear to be affected by different P supply to the RC. The internal P concentrations in plants, particularly in the root, may control the level of root colonization (Öpik et al., 2008). Garcia et al. (2007) established that root P concentration is negatively correlated to colonization. In the present experiment, P concentrations in the shoot were not consistently affected by P supply ratios in the RC, while P concentrations in belowground biomass were significantly affected by localised P supply in mycorrhizal roots only (Tab.3.3.B). The P concentration in the belowground biomass tended to be higher in RC that were supplied with the high amount of P. Menge et al. (1978) also studied on AM colonization in a split-root system and reported that high concentrations of soil P around part of the root system did not inhibit colonization of roots by AM fungi when the overall concentrations of P in the root system remained low.

Richardson et al. (2011) also suggested that total AM colonization per plant may not be decreased until soil P levels are very high. This may explain way a P rich patch does not always reduce AM fungal colonization of roots in this patch. However, in the present study, soil P supply levels were moderate, and a moderate soil P level is optimal for AM colonization (Olsson et al., 2006). Also, mycorrhizal colonization was measured only at the end of the experiment when much soil P may already have been taken up in the RC with relatively higher P supply. In addition, the fungal isolate used in this study may be particularly high P tolerant. Plant P status also regulates the rate of AM fungal P uptake (Nagy et al., 2009), so that the activity of AM fungal mycelium in P uptake as shown by the ratio of coarse (runner hyphae) to thin hyphae (absorbing hyphae) (Olsson et al., 2006) was not affected by different P supply ratio in the HC.

Even though the extent of root AM colonization was not affected by different P supply in the RC, the weight of mycelium and the hyphae length tended to be lower in HC that were supplied with a higher amount of P. Homogenously high levels of available P in soil usually reduce the development of external hyphae in soil (Olsson et al., 2006; Rakshit and Badhoria, 2008). The growth of external hyphae may even more decreased by a high level of soil P than root colonization, because roots may reduce the C flow to the fungus under improved P conditions. Under these conditions, intraradical mycelium reduces lipid transport to the extraradical mycelium (Olsson et al., 2002). Conversely, Nogueira and Cardoso (2006) reported that the extraradical fungal hyphae are not sensitive to high soil P levels.

The number of spores either per milligram mycelium or per meter hyphae length was also not significantly affected by the different P supply ratios to the HC. Satter et al. (2007) reported that spore populations in the soil show a similar trend as root colonization with different rates of P application. Douds and Schenk (1990) found that the sporulation of *G intraradices* was correlated with P concentration in the root and in the shoot of *Paspalum notatum*.

Nitrogen supply to soil has been shown to decrease, increase, or have no effect on AM colonization of roots, depending at least partly on P concentration in the soil. Arbuscular mycorrhizal colonization is increased with increased N supply when P is limited, but it is decreased when P is not limited (Johnson et al., 2003). In the present study, the extent of AM colonization and total weight of mycelium were not affected by N supply ratios to the sides of the split-root system. However, hyphae length and number of spores per milligram mycelium tended to be decreased in the HC that received a higher amount of N. Johnson et al. (2003)

reported that extraradical mycorrhizal structures (hyphae and spores) are more responsive to N supply than are intraradical structures. This response may be caused by decreasing photoassimilate allocation to the root exposed to higher N supply, leading to decreasing photosynthate supply to the fungus, and increasing N assimilation of the fungus (Wallenda et al., 1996).

In summary, the present study showed that

a. Dry matter of sweet potato plants was drastically increased by colonization of the root system with a mycorrhizal fungus, indicating a high mycorrhizal dependency of this plant species for adequate growth.
b. Mycorrhizal colonization also drastically increased plant P and N uptake.
c. Mycorrhizal colonization distinctly increased P concentrations in the shoot, indicating that increased P uptake may have been the main driver of the mycorrhizal effect on shoot growth.
d. Mycorrhizal colonization specifically increased the formation of tubers by sweet potato plants, and at the same time distinctly increased P, but not N concentrations in tubers.
e. Sweet potato shoot growth was not significantly affected by the spatial variation of P and N supply in soil, indicating a high ability of this plant species for nutrient translocation within the plant and nutrient integration for shoot growth.
f. The formation of tubers (but not of non-tuber roots) was distinctly increased in the soil zones with high P supply in soil in non-mycorrhizal but not in mycorrhizal plants, may be due to a stimulating signal for belowground growth, and
g. AM fungal biomass was relatively little influenced by local soil P and N supply, indicating that the AM fungus did not specifically forage for areas of high mineral P concentration in soil.

In conclusion, the present study indicated that mycorrhizal colonization may lead to decreased belowground plant responses to soil P patches. Model studies on effects of heterogeneous nutrient distribution in soil may result in misleading conclusion when performed with non-mycorrhizal plants or with plants of unknown mycorrhizal status.

A limitation of the present experiment is that only one fungal isolate was tested. Species and isolates of mycorrhizal fungi differ in their ability, for example, to grow extraradical mycelium and to contribute to host plant P uptake. It will be very interesting to

study in future experiments the response of plants associated with different AM fungal genotypes to heterogeneous nutrient distribution in soil (see Chapter 4 of this thesis).

4. THE RESPONSE OF SWEET POTATO PLANTS INOCULATED WITH DIFFERENT AM FUNGAL GENOTYPES TO HOMOGENEOUS AND HETEROGENEOUS PHOSPHORUS AND NITROGEN SUPPLY TO DIFFERENT PARTS OF THE ROOT

4.1 ABSTRACT

Under natural conditions, nutritional elements are usually not evenly spread throughout the soil. Plant roots can respond to nutrient rich patches in the soil with more or less flexibility in terms of both morphology and physiology. Most plants species in nature form mycorrhizal associations. Association with arbuscular mycorrhizal (AM) fungi may modify plant response to nutrient heterogeneity. However, species and strain of AM fungi have been shown to differ in the extent to which they increase nutrient uptake and growth of a given host plant species. In the present experiment, the response of plant growth to nutrient heterogeneity in soil was studied using sweet potato (*Ipomea batatas* L.). Plants were grown in split-root pots with different rates of either P or N fertilization, with the total amount of either P or N over the two root compartments of each split-root pot being similar. Plants were inoculated with isolates of either *Glomus mosseae* or *G. intraradices.* Mycorrhizal roots with both fungi responded to either P or N rich patches by root proliferation. However, total final root dry weight was not significantly different between heterogeneous and homogeneous P or N distribution in soil. The extraradical mycelium from both AM fungi did not actively forage for either P or N rich patches. Plants colonized by *G. intraradices* had slightly higher dry weight and tissue P and N concentrations than corresponding plants colonized by *G. mosseae.Glomus intraradices* showed higher AM root colonization and a lower ratio of coarse to thin hyphae than *G. mosseae*. I conclude that sweet potato plants respond to nutrient rich patches by root proliferation, and that AM fungi may increase plant nutrient uptake in the patch not by hyphal proliferation but by alteration of the capacity of the root or the hyphae to take up nutrients. *Glomus mosseae* was less effective in increasing nutrient uptake and growth of sweet potato plants compared with *G. intraradices*. These differences were related to the difference in the extent of AM colonization and particularly to the developmental pattern of the extraradical mycelium.

4.2 INTRODUCTION

Among the nutrients, phosphorus (P) and nitrogen (N) are required in large quantity by plants. Phosphorus is a structural element in nucleic acids, plays a key role in energy transfer as component of adenosine phosphate, and is also essential for transfer of carbohydrates in leaf cells. Nitrogen plays a central role in plant metabolism as a constituent of proteins, nucleic acids, chlorophyll, co-enzyms, phytohormones and secondary metabolites *(*Hawkesford et al., 2011). In natural soils, the distribution of nutrients is not homogeneous because of the uneven distribution of soil organic matter and the equally uneven rate of its

microbial decomposition. In agricultural soils, a heterogeneous nutrient distribution may be the result of an application of granular (at fine scale) and of banded (at coarse scales) fertilizer (Robinson et al., 1999).

Plant roots respond to a heterogeneous nutrient distribution in soil either with morphological (increasing root proliferation in the patch) or physiological (increasing the rate of nutrient uptake in the patch) mechanisms (Ma and Rengel, 2008). Most plant species form mycorrhizal associations (Smith and Read, 1997, p. 11). Arbuscular mycorrhizal (AM) fungi can forage for N or P in the patches by hyphal proliferation (Gavito and Olsson, 2008; Hodge and Fitter, 2010), so that mycorrhizal colonization can influence the activity of roots to forage for nutrients in the patch (Wijesinghe et al., 2001).

The role of the mycorrhizal symbiosis in contributing to acquisition of nutrients from a growth substrate where the distribution of P and N is not homogeneous is getting more attention recently (Hodge, 2009; see also Chapters 2 and 3 of this thesis). However, different arbuscular mycorrhizal fungi show differences in their potential to increase plant growth, related to differences in their nutrient uptake capacity (Mathur et al., 2006; Smith et al., 2003). The differences between AM fungi in their ability to increase plant performance depend on: (1) the ability of AM fungi to form extensive and well distributed hyphal networks in soil (Parniske, 2008); (2) the ability of AM fungi to rapidly spread infection over developing host root systems (Giovannetti, 2000); (3) the ability of extraradical hyphae to absorb P and other nutritional elements from soil solution (Neumann and George, 2009); (4) the fungal-plant host combination (Lovelock et al., 2003); and (5) the variation of AM fungal response to soil environmental conditions (Kelly et al., 2005).

It is known for a long time that soil conditions affect the ability of AM fungi to form symbiotic associations with host plants (Yanfang et al., 2012). The level of AM fungal root colonization was, for example, reduced in soil with high P concentration (Gabriel-Neumann et al., 2011). This effect is mainly caused by higher P concentration in roots under such conditions (Olsson et al., 2002).

The level of AM root colonization may be directly correlated with the length of extraradical hyphae in the soil, because the allocation of plant photosynthates to the fungus promotes hyphal growth inside and outside the root. Extraradical hyphae increase nutrient transfer from the soil to the plant (Lebrón et al., 2012). However, other observations suggest that the growth of extraradical hyphae may be more decreased at a high level of soil P than root colonization (Olsson et al., 2002), even though Nogueira and Cardoso (2006) reported that the extraradical fungal hyphae were not sensitive to high soil P level in their experiment.

The sporulation of AM fungi can also be affected by soil P concentration. Sporulation may be more abundant at lower soil P level (Lovelock et al., 2003). However, other observations suggest that the number of spores can be positively (Satter et al., 2007), negatively (Moreira et al., 2006), or not at all be correlated with the level of root colonization (Chandra and Kehri, 2008, p.221). Nitrogen supply to soil can decrease, increase, or not affect AM root colonization, depending on the P concentration in soil. AM colonization is increased at higher N supply when P is limited, but it is decreased in response to higher N supply when P is not limited. Furthermore, the extraradical structures (hyphae and spores) are more responsive to N supply than intraradical structures (Johnson et al., 2003).

The plant host, by its internal P status, regulates the AM fungal development (Scervino et al., 2005) and the activity of the fungal mycelium to forage for nutrients in the soil (Nagy et al., 2009). Higher P concentrations in the plant tissue, particularly in the roots, reduce the level of root colonization (Öpik et al., 2008), production of spores, and of secondary external hyphae (Grant et al., 2005). The activity of fungal mycelium can be related to the proportion of the ratio of coarse (runner) hyphae to thin (absorbing) hyphae. A higher proportion of absorbing hyphae than of runner hyphae indicated that these hyphae are active in P uptake (Olsson et al., 2006). The runner hyphae which have a wide diameter have a function in the rapid spread of the fungus from the living roots to the soil, while the absorbing hyphae have a function in increasing the availability of nutrients to the plant host (Peterson et al., 2004, p.71).

In the present experiment, the symbiotic performance of isolates of two AM fungal species, *Glomus mosseae* and *Glomus intraradices*, were investigated by assessing plant growth responses and P and N uptake in sweet potato (*Ipomea batatas*) plants grown in different P and N supply treatments in split-root pots. Isolates of both *G. mosseae* and *G. intraradices* were used as mycorrhizal inoculum in the present study because these AM fungi exhibit a different pattern of extraradical growth (Avio et al., 2006) and are different in the rate and extent of colonization (Jansa et al., 2008). Since most plants are AM fungi symbionts and nutrients in the soil are rarely homogeneously distributed, the present experiment addressed the following questions: (1) Does the extraradical AM mycelium from *G. mosseae* and *G. intraradices* actively forage for nutrients in the nutrient-rich soil patch? (2) How will AM fungal root colonization interact with root responses to the nutrient-rich soil patch?

4.3 MATERIALS AND METHODS

4.3.1 EXPERIMENTAL PLANT PREPARATION

Sweet potato (*Ipomea batatas*) motherplants were grown in nutrient solution containing 2.25 mM N (NH_4NO_3), 0.5 mM P (KH_2PO_4), 1.09 mM K (K_2SO_4 and KH_2PO_4), 2.71 mM Ca ($CaSO_4.2H_2O$), 2.71 mM S (K_2SO_4 and $CaSO_4.2H_2O$), 0.06 mM Fe (Fe-EDTA), 0.02 mM B (H_3BO_3), 4 µM Mn ($MnSO_4.H_2O$), 1.84 µM Zn ($ZnSO_4.7H_2O$), 3.15 µM Cu ($CuSO_4$), and 0.27 µM Mo ($(NH_4)_6Mo_7O_{24}.H_2O$). All other preparations were carried out as described in Chapter 3.3.1 of this thesis.

4.3.2 PREPARATION OF THE PLANTING POTS

See Chapter 3.3.2 of this thesis.

4.3.3 SET-UP OF THE INOCULATION AND FERTILIZATION TREATMENTS

At the start of the experiment, for the mycorrhizal treatments (+M) each RC of the split-roots pots was inoculated with 25 gram inoculum of *Glomus etunicatum*. The inoculum was a commercial product (AMyKor GmbH, Bitterfeld, Germany) based on expanded clay. The inoculum was mixed homogeneously with the soil before it was filled in the two RC of the split-root pots. For non-mycorrhizal treatments (-M), each RC was inoculated with 25 gram sterilized mycorrhizal inoculum and 60 ml of aqueous filtrate of inoculum to encourage a microflora similar to that in mycorrhizal treatments. Thereafter, the water content of the soil from both +M and –M were adjusted to approximately 17% w/w by addition of distillated water. The inoculum for –M treatments was sterilized by heating in the oven at 100°C overnight. The HC were not inoculated with AM fungal inoculums.

However, at 45 days after planting the roots for +M treatments were not colonized by the AM fungus, so that at 49 days after plating, the RC were inoculated with 30 gram inoculum of either *Glomus mosseae* (GM) or *Glomus intraradices* (GI)**.** Both *G. mosseae* and *G. intraradices* inoculum were obtained from pot cultures of the respective AM fungi with maize plants on the same C loess soil, and consisted of air-dried soil with extraradical AM mycelium, AM spores, and colonized root fragments. The inoculum was inserted into six holes with a diameter of 5 mm. The holes extended to the base of the pot and surrounded the plant. The HC were not supplied with fungal inoculum.

All plants were also supplied with additional100 mg P and 300 mg N in total. The mode, by which these total amounts of N and P were distributed over the two compartments

of each split-root pot, differed depending on the treatment. In treatments with homogeneous nutrient supply, both adjacent compartments were supplied with 50 mg P kg^{-1} dry soil (50:50) and 150 mg N kg^{-1} dry soil (150:150). In the P gradient treatments, the P supply level in split-root pots was either (mg kg^{-1} dry soil) 70:30 or 85:15. In these treatments, the N supply level was 150:150. In the N gradient treatments, the P supply level was 50:50, while the amount of N was (mg kg^{-1} dry soil) 180:120, 210:90 or 255:45. The fertilization of the soil in the HC corresponded to that of the respective RC. At 13 days after inoculation (62 days after planting), the soil in all RC was fertilized with an additional 30% of the initially applied P (in form of KH_2PO_4) and 100% of the initially applied N (in form of NH_4NO_3). K fertilization between the treatments was balanced with additional K_2SO_4 to obtain 17% of initially applied K in all treatments. The position of the HC in the experimental split-root pot can be seen in Fig. 3.1. (Chapter 3 of this thesis).

4.3.4 PLANT GROWTH CONDITIONS

The pots were set up completely randomized in a greenhouse in Grossbeeren (long. 13º20'E; lat. 51º22'N), Germany, for sixteen weeks from 28 August 2008 to 21 December 2008 with a light period of 12 h day/12 h night. Average light intensity was 960 µmol m^{-2} s^{-1} during the day, and there was not additional artificial light supply. Average air temperature in the glasshouse during this time was 23 ºC day/20 ºC night, and relative humidity averaged 55%. All planting pots of this experiment changed their position on the planting table at regular intervals, but a completely randomized design was maintained. The gravimetric water content of the soil was adjusted to approximately 17% w/w after the plants were inserted. Water loss from the pots was estimated gravimetrically, and was replaced by deionized water every two days. Irrigation water was distributed over the two RC of each split-root pot according to visual appraisal.

4.3.5 HARVEST AND ANALYSIS OF PLANT AND ARBUSCULAR MYCORRHIZAL FUNGAL MATERIAL

See Chapter 3.3.5 of this thesis.

4.3.6 STATISTICAL ANALYSIS

The experiment had a completely randomized design with four replicates per treatment. Treatment effects were statistically analyzed by SPSS (SPSS 15, SPSS Inc. Chicago, USA). A Two-Way ANOVA was conducted to assess whether the fertilization

treatments and the identity of the AM fungus had a significant effect on the mean values of plant growth and nutrient uptake parameters. A Duncan Multiple Range Test was conducted to identify significant differences between the mean values. In all tests, differences were considered significant when $P<0.05$. In addition, a correlation analysis was conducted to identify the relationship between total hyphae length of either *G. mosseae* or *G. intraradices* in split-root pots that received P or N fertilization treatments and plant P or N uptake, respectively.

4.4 RESULTS

4.4.1 PLANT DRY WEIGHT AFTER HARVEST

The total plant and shoot DW of plants inoculated with either *G. mosseae* or *G. intraradices* was not affected by either P or N supply treatments (Tabs. 4.1.A and 4.1.D).However, plants inoculated with *G. intraradices* had a higher plant and shoot DW compared with plants inoculated with *G. mosseae*, particularly in the P supply treatments (Tab. 4.1.A).

The total root DW of plants inoculated with either *G. mosseae* or *G. intraradices* was also not significantly affected by either P or N supply treatments (Tabs. 4.1.A and 4.1.D). The total root DW of plants was not significantly different between plants inoculated with *G. mosseae* and with *G. intraradices* in both nutrient distribution treatments. However, the ratio of the root DW of the two halves of the root system of plants inoculated with either *G. mosseae* or *G. intraradices* was affected by both P or N supply treatments (Tabs. 4.1.A and 4.1.D). In plants inoculated with either *G. mosseae* or *G. intraradices*, root DW tended to be increased in the RC that received a higher amount of P (Tabs. 4.1.B and 4.1.C). The root DW also tended to be higher in the RC that received a higher amount of N, particularly in plants inoculated with *G. mossseae* (Tabs. 4.1.E and 4.1.F). Plants inoculated with *G. mosseae* had a higher ratio of root DW of two halves of the root system than plants inoculated with *G. intraradices*, particularly in the N supply treatments (Tab. 4.1.D).

Tuber DW of plants inoculated with either *G. mosseae* or *G. intraradices* was significantly affected by P supply treatments, but not by N supply treatments (Tabs. 4.1.A and 4.1.D). Total tuber dry weight of plants inoculated with either *G. mosseae* or *G. intraradices* tended to increase with increasing P supply ratio. Tuber dry weight tended to be increased in the RC that received the lower amount of P supply, particularly in plants

inoculated with *G. intraradices* (Tabs. 4.1.A., 4.1.B and 4.1.C). Tuber growth was very variable between replications, resulting in high standard deviations of means. There was no significance difference between plants inoculated with *G. mosseae* and with *G. intraradices* in the production of tubers in either P or N supply treatments (Tabs. 4.1.A and 4.1.D).

Table 4.1.A: Total plant DW, shoot DW, root and tuber DW, shoot/root ratio, aboveground/belowground ratios as well as ratio of DW of the two halves (root compartments, RC) of the split-root system of plants exposed to different P supply treatments and inoculated either with *G. mosseae* (GM) or *G. intraradices*. A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction is also given. In case the ANOVA indicated a significant effect of the P supply ratio, a post-hoc Duncan's Multiple Range Test (DMRT) was performed to test how the mean values among the different P supply ratio treatments differ.

P supply ratio to the two halves of the split-pot system (RCs+HCs)		50:50	70:30	85:15	Statistical significances			DMRT for P supply ratio
					AM fungal strain	P supply ratio	Inter-action	
Total plant DW (g per plant)	GM	16.41ab ± 2.73	17.62ab ± 2.01	15.29a ± 2.29	*	ns	ns	-
	GI	18.99ab ± 1.22	18.35ab ± 4.07	20.49b ± 1.82				
Shoot DW (g per plant)	GM	7.26ab ± 1.97	6.35ab ± 1.14	5.33a ± 1.80	*	ns	ns	-
	GI	10.07b ± 3.96	7.88ab ± 2.17	8.76ab ± 2.80				
Root DW in the two RCs (g per plant)	GM	2.43 ± 0.80	1.92 ± 0.22	1.58 ± 0.57	ns	ns	ns	-
	GI	2.73 ± 0.80	2.21 ± 0.55	2.19 ± 0.49				
Tuber DW in the two RCs (g per plant)	GM	6.73a ± 1.83	9.35a ± 1.12	8.38a ± 1.18	ns	*	ns	15:85,30:70 >50;50
	GI	6.19a ± 3.72	8.27a ± 1.45	9.54a ± 2.10				
Shoot/root ratio	GM	3.03 ± 0.20	3.37 ± 0.84	3.40 ± 0.19	ns	ns	ns	-
	GI	3.64 ± 0.64	3.58 ± 0.38	3.94 ± 0.55				
Aboveground/ belowground ratio	GM	0.81 ± 0.28	0.56 ± 0.10	0.54 ± 0.19	ns	ns	ns	-
	GI	1.41 ± 1.18	0.75 ± 0.09	0.78 ± 0.36				
Ratio of root DW of the two halves of the root systems	GM	0.82a ± 0.09	1.08b ± 0.05	1.15b ± 0.13	ns	*	ns	15:85,30:70 >50:50
	GI	0.82a ± 0.08	1.07b ± 0.15	1.03b ± 0.20				
Ratio of belowground DW of the two halves of the root system	GM	0.40 ± 0.12	0.68 ± 0.45	2.06 ± 1.96	ns	ns	ns	-
	GI	1.05 ± 1.16	0.87 ± 0.34	1.05 ± 0.99				

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 4.1.B: Root and tuber DW in the compartments (RC) of the split-root pot that received the lower amount of P. For further explanation, see Tab. 4.1.A.

P supply (mg kg^{-1})		50	30	15	Statistical significances		
					AM fungal strain	P supply	Inter-action
Root DW (g per RC)	GM	1.21 ± 0.40	0.88 ± 0.13	0.71± 0.35	ns	ns	ns
	GI	1.36 ± 0.40	1.01 ± 0.15	1.11 ± 0.42			
Tuber DW (g per RC)	GM	3.36 ± 0.92	7.04 ± 3.47	3.39 ± 3.32	ns	ns	ns
	GI	3.10 ± 1.86	5.10 ± 2.43	5.89 ± 4.07			

Table 4.1.C: Root and tuber DW in the compartments (RC) of the split-root pot that received the higher amount of P. For further explanation, see Tab. 4.1.A.

P supply (mg kg^{-1})		50	70	85	Statistical significances		
					AM fungal strain	P supply	Inter-action
Root DW (g per RC)	GM	1.21 ± 0.40	1.03 ± 0.10	0.87 ± 0.22	ns	ns	ns
	GI	1.36 ± 0.40	1.19 ± 0.44	1.08 ± 0.13			
Tuber DW (g per RC)	GM	3.36 ± 0.92	2.31 ± 2.70	5.00 ± 3.77	ns	ns	ns
	GI	3.10 ± 1.86	3.17 ± 2.25	3.65 ± 4.09			

Table 4.1.D: The total plant DW, shoot DW, root and tuber DW, shoot/root ratio, aboveground/belowground ratio as well as ratio of DW of the two halves (root compartments, RC) of the split-root system of plants exposed to different N supply treatments and inoculated either with *G. mosseae* or *G. intraradices*. For further explanation, see Tab. 4.1.A.

N supply ratio to the two halves of the split-pot system (RCs+HCs)		150:150	180:120	210:90	255:45	Statistical significances		
						AM fungal strain	N supply ratio	Inter-action
Total plant DW (g per plant)	GM	16.41 ± 2.73	13.69 ± 2.47	15.10 ± 4.44	19.12 ± 2.61	ns	ns	ns
	GI	18.99 ± 1.22	18.02 ± 0.97	17.27 ± 3.20	15.58 ± 2.17			
Shoot DW (g per plant)	GM	7.26 ± 1.97	5.59 ± 1.91	6.95 ± 3.28	8.04 ± 2.04	ns	ns	ns
	GI	10.07 ± 3.96	9.70 ± 3.74	8.48 ± 4.51	7.00 ± 1.83			
Root DW in the two RCs (g per plant)	GM	2.43 ± 0.80	1.79 ± 0.62	2.07 ± 0.99	2.34 ± 0.50	ns	ns	ns
	GI	2.73 ± 0.80	2.79 ± 0.57	2.60 ± 1.07	2.15 ± 0.64			
Tuber DW in the two RCs (g per plant)	GM	6.73 ±1.83	6.30 ± 0.49	6.08 ± 1.34	8.73 ± 0.87	ns	ns	ns
	GI	6.19 ± 3.72	5.52 ± 3.38	6.18 ± 4.06	6.43 ± 1.69			
Shoot root ratio	GM	3.03 ± 0.20	3.12 ± 0.23	3.35 ± 0.21	3.43 ± 0.34	ns	ns	ns
	GI	3.64 ± 0.64	3.40 ± 0.63	3.15 ± 0.41	3.28 ± 0.31			
Aboveground/ belowground ratio	GM	0.81 ± 0.28	0.68 ± 0.19	0.84 ± 0.30	0.73 ± 0.18	ns	ns	ns
	GI	1.41 ± 1.18	1.51 ± 1.37	1.28 ± 1.31	0.83 ± 0.26			
Ratio of root DW of the two halves of the root systems	GM	0.82a ± 0.09	1.25c ± 0.17	1.05ab ± 0.17	1.10bc ± 0.20	*	*	ns
	GI	0.82a ± 0.08	0.88ab ± 0.16	0.93ab ± 0.06	1.12bc ± 0.25			
Ratio of belowground DW of the two halves of the root system	GM	0.40a ± 0.12	3.33b ± 0.48	0.70ab ± 0.60	0.59ab ± 0.36	ns	*	*
	GI	1.05ab ± 1.16	0.58ab ± 0.47	0.58ab ± 0.28	1.86b ± 1.67			

Table 4.1.E: Root and tuber DW in the compartments (RC) of the split-root pot that received the lower amount of N. For further explanation, see Tab. 4.1.A.

N supply (mg kg^{-1})		150	120	90	45	Statistical significances		
						AM fungal strain	N supply	Inter-action
Root DW (g per RC)	GM	1.21ab ± 0.46	0.70a ± 0.24	0.98ab ± 0.43	1.05ab ± 0.13	*	ns	ns
	GI	1.36b ± 0.46	1.55b ± 0.10	1.39b ± 0.56	0.97ab ± 0.36			
Tuber DW (g per RC)	GM	3.36abc ± 0.92	0.00a ± 0.00	5.15bc ± 2.82	7.20c ± 2.28	ns	ns	*
	GI	3.10ab ± 1.86	5.34bc ± 3.72	5.19bc ± 3.15	2.6ab ± 2.94			

Table 4.1.F: Root and tuber DW in the compartments (RC) of the split-root pot that received the higher amount of N. For further explanation, see Tab. 4.1.A.

N supply (mg kg^{-1})		150	180	210	255	Statistical significances		
						AM fungal strain	N supply	Inter-action
Root DW (g per RC)	GM	1.21 ± 0.46	1.09 ± 0.41	1.10 ± 0.59	1.30 ± 0.45	ns	ns	ns
	GI	1.36 ± 0.46	1.24 ± 0.50	1.21 ± 0.52	1.18 ± 0.41			
Tuber DW (g per RC)	GM	3.36abc ± 0.92	6.30c ± 0.49	0.92ab ± 1.59	1.54ab ± 2.26	ns	ns	*
	GI	3.10abc ± 1.86	0.18a ± 0.36	1.00ab ± 1.92	3.81bc ± 4.44			

Shoot/root and aboveground/belowground ratios of plants inoculated with either *G. mosseae* or *G. intraradices* were not significantly affected by either P or N supply treatments. There was no significance difference between *G. mosseae* and *G. intraradices* in the effect on shoot/root and aboveground/belowground ratio in either P or N supply treatments (Tabs. 4.1.A and 4.1.D). The ratio of belowground DW of the two halves of the root system of plants inoculated with either *G. mosseae* or *G. intraradices* was not significantly affected by the P supply treatments. There was also no significant difference between plants inoculated with *G. mosseae* and with *G. intraradices* in the ratio of belowground DW of the two halves of the root system (Tab. 4.1.A). In contrast, the ratio of belowground DW of the two halves of the root system of plants inoculated with *G. mosseae* was increased at increasing N supply ratio, particularly at the 180:120 N distribution level (Tab. 4.1.D). This effect is related to a higher root and tuber dry weight in the RC that

received the higher amount of N compared with the RC that received the lower amount of N (Tabs. 4.1.E and 4.1.F).

4.4.2 THE ARBUSCULAR MYCORRHIZAL FUNGAL COLONIZED ROOT LENGTH AND THE AMOUNT OF MYCELIUM OBTAINED FROM THE FUNGAL COMPARTMENTS

The ratios of AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spore per mg mycelium, and of number of spores per m hyphae length of the two parts of the split-root system from both *G. mosseae*and *G. intraradices* were not significantly affected by either P or N supply treatments. There were no significant differences between *G. mosseae* and *G. intraradices* in the ratios of AM fungal development of the two sides (RC+HC) of the split-root system (Tabs. 4.2.A and 4.2.D).

Table 4.2.A: Ratios of AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spore per mg mycelium, and of number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots exposed to different P supply treatments and inoculated either with *G. mosseae* or *G. intraradices*. A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction is also given.

P supply ratios in the two sides (RC+HC) of the split-root pots		50:50	70:30	85:15	Statistical significances		
					AM Fungal strain	P supply ratio	Inter-action
AM colonization (%)	GM	1.21b ± 0.25	1.04ab ± 0.23	0.85a ± 0.08	ns	ns	*
	GI	0.90a ± 0.09	1.12ab ± 0.15	1.12ab ± 0.15			
Hyphae length (m/cm^3 soil)	GM	1.35 ± 0.75	1.06 ± 0.92	1.51 ± 1.11	ns	ns	ns
	GI	1.71 ± 0.55	0.69 ± 0.25	0.95 ± 0.14			
Ratio coarse to thin hyphae	GM	1.36 ± 0.22	0.89 ± 0.39	0.73 ± 0.60	ns	ns	ns
	GI	1.12 ± 0.20	1.11 ± 0.43	1.02 ± 0.07			
Weight of mycelium (mg per HC)	GM	1.46 ± 0.79	1.22 ± 0.91	1.86 ± 1.59	ns	ns	ns
	GI	1.80 ± 0.45	0.71 ± 0.26	1.11 ± 0.16			
Number of spore per mg mycelium	GM	1.01 ± 0.29	0.90 ± 0.30	1.04 ± 0.22	ns	ns	ns
	GI	1.24 ± 0.38	1.11 ± 0.37	1.01 ± 0.33			
Number of spores per m hyphae length	GM	0.93 ±0.59	1.30 ± 0.91	1.26 ± 1.27	ns	ns	ns
	GI	0.59 ± 0.19	1.72 ± 0.70	1.06 ± 0.30			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 4.2.B: The AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spore per mg mycelium, and of number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots that received the lower amount of P. For further explanation, see Tab. 4.2.A.

P supply (mg kg^{-1})		50	30	15	Statistical significances		
					AM Fungal strain	P supply	Inter-action
AM colonization (%)	GM	37.99bc ± 14.08	28.72ab ± 12.33	19.85a ± 11.91	*	*	ns
	GI	49.02c ± 6.97	50.38 c ± 4.15	40.04bc ± 9.31			
Hyphae length (m/cm^3 soil)	GM	1.95 ± 2.25	2.52 ± 2.97	2.22 ± 1.55	ns	ns	ns
	GI	3.99 ± 3.89	3.96 ± 1.07	4.17 ± 2.09			
Ratio coarse to thin hyphae	GM	0.93ab ± 0.35	1.43bc ± 0.89	2.11c ± 0.66	*	ns	ns
	GI	0.36a ± 0.17	0.25a ± 0.09	0.33a ± 0.07			
Weight of mycelium (mg per HC)	GM	1.15 ± 0.73	2.84 ± 3.64	1.25 ± 0.78	ns	ns	ns
	GI	4.78 ± 4.97	2.81 ± 0.67	3.10 ± 1.77			
Number of spores per mg mycelium	GM	2124ab ± 395	1881a ± 615	1747a± 439	*	ns	ns
	GI	3142ab ± 1023	2920ab ± 1365	3495b ±1029			
Number of spores per m hyphae length	GM	119.95 ± 110.26	137.61a ± 161.90	69.00 ± 83.02	ns	ns	ns
	GI	81.31 ± 44.72	33.05 ± 18.73	39.84 ± 18.73			

Table 4.2.C: The AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spore per mg mycelium, and of number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots that received the higher amount of P. For further explanation, see Tab. 4.2.A.

P supply (mg kg-1)		50	70	85	Statistical significances		
					AM Fungal strain	P supply	Inter-action
AM colonization (%)	GM	37.99bc ± 14.08	27.76ab ± 2.62	19.92a ± 6.07	*	ns	ns
	GI	49.02c ± 6.97	50.96c ± 10.29	48.32c ± 1.73			
Hyphae Length (m/cm^3 soil)	GM	1.95 ± 2.25	1.13 ± 0.68	2.97 ± 1.83	ns	ns	ns
	GI	3.99 ± 3.89	1.79 ± 0.72	3.51 ± 1.02			
Ratio coarse to thin hyphae	GM	0.93ab ± 0.35	0.89ab ± 0.38	2.31b ± 2.55	*	ns	ns
	GI	0.36a ± 0.17	0.28a ± 0.12	0.34a ± 0.07			
Weight of mycelium (mg per HC)	GM	1.15 ± 0.73	1.22 ± 0.51	2.60 ± 1.74	ns	ns	ns
	GI	4.78 ± 4.97	1.47 ± 0.94	3.49 ± 1.39			
Number of spore per mg mycelium	GM	2124ab ± 395	1409a ± 578	1707a ± 434	*	ns	ns
	GI	3142b ± 1023	3111b ± 819	3377b ±1432			
Number of spores per m hyphae length	GM	119.95 ± 110.26	69.67 ± 41.89	45.65 ± 54.96	ns	ns	ns
	GI	81.31 ± 44.72	79.65 ± 28.49	11.40 ± 8.18			

Table 4.2.D: Ratios of AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spore per mg mycelium, and of number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots exposed to different N supply treatments. For further explanation, see Tab. 4.2.A.

N supply ratio in the two sides (RC+HC) of the split-root pots		150:150	180:120	201:90	255:45	Statistical significances		
						AM Fungal strain	N supply ratio	Inter-action
AM colonization (%)	GM	1.21 ± 0.25	0.97 ± 0.09	0.87 ± 0.30	1.12 ± 0.75	ns	ns	ns
	GI	0.90 ± 0.09	0.76 ± 0.04	0.94 ± 0.04	0.92 ± 0.14			
Hyphae length (m/cm^3 soil)	GM	1.35 ± 0.75	1.39 ± 0.43	2.27 ± 1.88	2.30 ± 3.08	ns	ns	ns
	GI	1.71 ± 0.55	1.63 ± 0.46	0.96 ± 0.29	0.95 ± 0.33			
Ratio coarse to thin hyphae	GM	1.36 ± 0.22	1.04 ± 0.33	0.87 ± 0.31	1.18 ± 0.38	ns	ns	ns
	GI	1.12 ± 0.20	1.08 ± 0.24	1.17 ± 0.47	1.28 ± 0.16			
Weight of mycelium (mg per HC)	GM	1.46 ± 0.79	1.23 ± 0.36	1.72 ± 1.38	1.62 ± 1.58	ns	ns	ns
	GI	1.80 ± 0.45	1.87 ± 0.87	0.90 ± 0.31	0.76 ± 0.14			
Number of spores per mg mycelium	GM	1.01 ± 0.29	1.05 ± 0.18	1.34 ± 0.42	1.10 ± 0.27	ns	ns	ns
	GI	1.24 ± 0.38	0.79 ± 0.13	0.87 ± 0.23	1.01 ± 0.40			
Number of spores per m hyphae length	GM	0.93 ± 0.59	0.85 ± 0.41	1.2 ± 1.32	1.29 ± 0.85	ns	ns	ns
	GI	0.59 ± 0.19	0.52 ± 0.21	0.93 ± 0.11	1.22 ± 0.87			

Table 4.2.E AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spore per mg mycelium, and of number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots that received the lower amount of N. For further explanation, see Tab. 4.2.A.

N supply (mg kg^{-1})		150	120	90	45	Statistical significances		
						AM Fungal strain	N supply	Inter-action
AM colonization (%)	GM	37.99abc ± 14.08	33.21a ± 3.51	34.83ab ± 10.91	39.80abc ± 21.46	*	ns	ns
	GI	49.02abc ± 6.97	52.77bc ± 5.69	54.93c ± 13.89	50.54abc ± 6.65			
Hyphae length (m/cm^3 soil)	GM	1.95 ± 2.25	3.32 ± 1.81	2.45 ± 1.56	5.56 ± 5.12	ns	ns	ns
	GI	3.99 ± 3.89	4.75 ± 2.99	5.00 ± 3.09	6.78 ± 4.02			
Ratio coarse to thin hyphae	GM	0.93ab ± 0.35	1.30b ± 0.59	1.59b ± 0.86	0.53b ± 0.32	*	ns	ns
	GI	0.36a ± 0.17	0.28a ± 0.15	0.39a ± 0.09	0.42a ± 0.40			
Weight of mycelium (mg per HC)	GM	1.15a ± 0.73	2.50ab ± 1.02	2.80ab ± 2.42	3.34ab ± 2.56	*	ns	ns
	GI	4.78ab ± 4.97	3.77ab ± 2.96	5.07ab ± 3.78	6.84b ± 1.97			
Number of spore per mg mycelium	GM	2124ab ± 395	2055ab ± 242	1522a ± 536	2011ab ± 830	*	ns	ns
	GI	3142abc ± 1023	3763bc ± 855	3470bc ± 916	3992c ± 2477			
Number of spores per m hyphae length	GM	119.95b ± 110.26	30.54a ± 12.24	55.31ab ± 70.13	10.94a ± 5.57	ns	*	ns
	GI	81.31ab ± 44.72	39.84a ± 18.73	40.26ab ± 31.60	30.23a ± 15.66			

Table 4.2.F: The AM root colonization, hyphae length, ratio of coarse to thin hyphae, weight of mycelium, number of spore per mg mycelium, and of number of spores per m hyphae length of the two sides (RC+HC) of the split-root pots that received the higher amount of N. For further explanation, see Tab. 4.2.A.

N supply (mg kg^{-1})		150	180	210	255	Statistical significance		
						AM Fungal strain	N supply	Inter-action
AM colonization (%)	GM	37.99abc ± 14.08	30.84ab ± 4.14	24.91a ± 11.06	32.03ab ± 9.80	*	ns	ns
	GI	49.02c ± 6.97	36.27ab ± 4.49	48.71b ± 13.57	42.64bc ± 9.89			
Hyphae length (m/cm^3 soil)	GM	1.95 ± 2.25	5.70 ± 2.31	6.57 ± 4.37	3.55 ± 2.14	ns	ns	ns
	GI	3.99 ± 3.89	12.73 ± 8.39	5.57 ± 5.30	6.07 ± 1.55			
Ratio coarse to thin hyphae	GM	0.93cd ± 0.35	1.22d ± 0.26	0.85bcd ± 0.17	0.86bcd ± 0.67	*	ns	ns
	GI	0.36ab ± 0.17	0.29a ± 0.04	0.51abc ± 0.27	0.37ab ± 0.14			
Weight of mycelium (mg per HC)	GM	1.15a ± 0.73	3.48a ± 1.13	2.97a ± 2.23	2.52a ± 1.17	*	ns	ns
	GI	4.78a ± 4.97	14.40b ± 13.33	6.03a ± 7.73	3.80a ± 0.88			
Number of spore per mg mycelium	GM	2124 ± 395	2345 ± 907	2423 ± 533	2242 ± 467	ns	ns	ns
	GI	3142 ± 1023	2309 ± 661	2917 ± 1641	3400 ± 570			
Number of spores per m hyphae length	GM	119.95 ± 110.26	19.78 ± 11.79	27.29 ± 27.46	34.08 ± 21.61	ns	ns	ns
	GI	81.31 ± 44.72	11.40 ± 8.18	36.48 ± 28.69	24.92 ± 10.25			

However, the rate of AM root colonization of plants inoculated with *G. mosseae* tended to be decreased in the RC that received either the higher or the lower amount of P because the 50:50 treatment had the highest colonization (Tabs. 4.2.B and 4.2.C). *Glomus intraradices* had significantly higher AM root colonization and number of spores per mg weight of mycelium than *G. mosseae* in all P or N supply treatments (Tabs. 4.2.B, 4.2.C, 4.2.E, and 4.2.F). *Glomus intraradices* also had a higher weight of mycelium than *G. mosseae* in the N supply treatments (Tabs. 4.2.E. and 4.2.F), while the weight of mycelium from both fungi was not significantly different in the P supply treatments (Tabs. 4.2.B and 4.2.C).

Hyphae length and number of spores per m hyphae length from both AM fungi were not significantly affected by P supply treatments (Tabs. 4.2.B, 4.2.C). There was no

significant difference between *G. mosseae* and *G. intraradices* in hyphae length and number of spore per m hyphae length in either P or N supply treatment (Tabs. 4.2.B, 4.2.C, 4.2.E, and 4.2.F). However, the number of spores per m hyphae length from both AM fungi was decreased in the HCs that received a lower amount of N (Tab. 4.2.E). The ratio of coarse to thin hyphae of *G. mosseae* was higher compared to the same ratio in *G. intraradices* in all P or N supply treatments. The ratio of coarse to thin hyphae in both fungi was not significantly affected by either P or N supply treatment (Tabs. 4.2.B, 4.2.C, 4.2.E and 4.2.F).

4.4.3. PHOSPHORUS AND NITROGEN PLANT CONCENTRATIONS AND TOTAL PLANT PHOSPHORUS AND NITROGEN UPTAKE AT DIFFERENT PHOSPHORUS SUPPLY

The plant P and N content, and shoot P and N concentrations of plants inoculated with either *G. mosseae* or *G. intraradices* were not significantly affected either P or N supply treatments (Tabs. 4.3.A and 4.3.D). However, plants inoculated with *G. intraradices* had significantly higher plant P content and shoot P concentrations compared with plants inoculated with *G. mosseae* in both P and N supply treatments (Tabs. 4.3.A and 4.3.D).

Table 4.3.A: Total P content, P concentration in the shoot, total N content, and N concentration in the shoot of plants exposed to different P supply treatments and inoculated either with *G. mosseae* or *G. intraradices*. A significant ($P<0.05$) effect of these main factors is indicated by a star. Significant interaction is also given.

P supply ratio to the two halves of the split-pot system (RCs+HCs)		50:50	70:30	85:15	Statistical significances		
					AM fungal strain	P supply ratio	Inter-action
Total P content (mg per plant)	GM	19.62a ± 4.53	16.19a ± 1.14	13.90a ± 1.39	*	ns	ns
	GI	28.78b ± 6.20	26.34b ± 3.70	27.53b ± 2.87			
P concentration in the shoot (mg g^{-1} DW)	GM	1.30a ± 0.14	1.20a ± 0.22	1.25a ± 0.31	*	ns	ns
	GI	1.78a ± 0.10	1.93a ± 0.43	1.60ab ± 0.12			
Total N content (mg per plant)	GM	328.34 ± 65.97	317.48 ± 37.77	266.80 ± 54.96	ns	ns	ns
	GI	338.64 ±102.61	344.53 ± 81.65	368.74 ± 47.06			
N concentration in the shoot (mg g^{-1} DW)	GM	22.73 ± 1.70	21.29 ± 1.35	20.75 ± 2.09	ns	ns	ns
	GI	22.60 ± 2.71	24.24 ± 2.47	21.88 ± 2.42			

Values are means and SD. Mean values followed by the same letter within the same parameter are not significantly ($P<0.05$) different.

Table 4.3.B: Total P content, P concentration, total N content, and N concentration in the belowground biomass in the RC of the split-root pot that received the lower amount of P. For further explanation, see Tab. 4.3.A.

P supply (mg kg^{-1})		50	30	15	Statistical significances		
					AM fungal strain	P supply	Inter-action
P content in the root and tuber (mg per RC)	GM	5.13 ± 1.50	6.03 ± 1.89	2.98 ± 2.30	ns	ns	ns
	GI	5.53 ± 1.96	6.62 ± 3.74	7.46 ± 5.29			
P concentration in the root and tuber (mg g^{-1} DW)	GM	1.10ab ± 0.12	0.80a ± 0.14	0.83a ± 0.26	*	*	ns
	GI	1.41ab ± 0.38	1.05ab ± 0.26	0.98a ± 0.21			
N content in the root and tuber (mg per RC)	GM	81.69 ± 18.19	129.80 ± 51.92	89.92 ± 56.59	ns	ns	ns
	GI	62.57 ± 28.14	100.30 ± 29.10	107.36 ± 55.99			
N concentration in the root and tuber (mg g^{-1} DW)	GM	21.63b ± 2.47	16.60a ± 0.74	17.28ab ± 2.93	ns	*	ns
	GI	21.58b ± 5.05	16.95ab ± 2.14	16.46a ± 2.68			

Table 4.3.C: Total P content, P concentration, total N content, and N concentration in the belowground biomass in the RC of the split-root pot that received the higher amount of P. For further explanation, see Tab. 4.3.A.

P supply (mg kg^{-1})		50	70	85	Statistical significances		
					AM fungal strain	P supply	Inter-action
P content in the root and tuber (mg per RC)	GM	5.13a ± 1.50	2.70a ± 1.94	4.62a ± 2.27	ns	ns	ns
	GI	5.53a ± 1.96	5.21a ± 3.18	6.27a ± 4.57			
P concentration in the root and tuber (mg g^{-}1 DW)	GM	1.10abc ± 0.12	0.93ab ± 0.22	0.90a ± 0.24	*	ns	ns
	GI	1.41bc ± 0.38	1.18abc ± 0.21	1.55c ± 0.52			
N content in the root and tuber (mg RC)	GM	81.69a ± 18.19	53.62a ± 32.11	91.18a ± 51.99	ns	ns	ns
	GI	62.5a ± 28.14	76.26a ± 28.13	72.99a ± 46.94			
N concentration in the root and tuber (mg g^{-1} DW)	GM	21.63a ± 2.47	20.36a ± 7.02	17.59a ± 5.45	ns	ns	ns
	GI	21.58a ± 5.05	18.59a ± 5.88	19.09a ± 7.18			

Table 4.3.D: Total P content, P concentration in the shoot, total N content, and N concentration in the shoot of plants exposed to different N supply treatments and inoculated either with *G. mosseae* or *G. intraradices.* For further explanation, see Tab. 4.3.A.

N supply ratio to the two halves of the split-pot system (RCs+HCs)		150:150	180:120	210:90	255:45	Statistical significances		
						AM fungal strain	N supply ratio	Inter-action
Total P content (mg per plant)	GM	19.62ab ± 4.53	15.57a ± 3.34	17.71a ± 2.26	18.42a ± 1.70	*	ns	ns
	GI	28.78c ± 6.20	31.92c ± 4.12	26.63bc ± 7.21	26.08bc ± 6.93			
P concentration in the shoot (mg g^{-1} DW)	GM	1.30ab ± 0.14	1.48bc ± 0.21	1.53bc ± 0.49	1.03a ± 0.24	*	ns	ns
	GI	1.78cd ± 0.10	2.13d ± 0.26	1.98d ± 0.21	1.95d ± 0.17			
Total N uptake (mg per plant)	GM	328.34 ± 65.97	261.43 ± 64.17	284.74 ± 64.51	334.25 ± 37.53	ns	ns	ns
	GI	338.64 ± 102.61	404.82 ± 74.52	335.57 ± 72.64	316.86 ± 55.91			
N concentration in the shoot (mg g^{-1} DW)	GM	22.73abc ± 1.70	22.88abc ± 1.63	22.22ab ± 4.34	19.96a ± 1.96	*	ns	ns
	GI	22.60abc ± 2.71	26.36c ± 1.62	22.84abc ± 2.76	24.27bc ± 1.21			

Table 4.3.E: Total P content, P concentration, total N content, and N concentration in the belowground biomass in the RC of the split-root pot that received the lower amount of N. For further explanation, see Tab. 4.3.A.

N supply (mg kg^{-1})		150	120	90	45	Statistical significances		
						AM fungal strain	N supply	Inter-action
P content in the root and tuber (mg per RC)	GM	5.13ab ± 1.50	0.90a ± 0.52	5.88ab ± 3.76	7.26b ± 3.30	ns	ns	*
	GI	5.53ab ± 1.96	9.39b ± 4.95	7.10b ± 2.67	5.78ab ± 5.55			
P concentration in the root and tuber (mg g^{-1} DW)	GM	1.10ab ± 0.12	1.23ab ± 0.46	0.90a ± 0.24	0.85a ± 0.17	*	ns	ns
	GI	1.41b ± 0.38	1.43b ± 0.22	1.10ab ± 0.12	1.55b ± 0.37			
N content in the root and tuber (mg per RC)	GM	81.69bc ± 18.19	22.96a ± 5.01	93.04bc ± 41.18	113.84c ± 36.46	ns	ns	*
	GI	62.57abc ± 28.14	107.07bc ± 45.42	99.13bc ± 27.20	53.72ab ± 41.17			
N concentration in the root and tuber (mg g^{-1} DW)	GM	21.63b ± 2.47	29.69c ± 1.47	16.05ab ± 2.39	13.69a ± 1.19	ns	*	*
	GI	21.58b ± 5.05	18.40ab ± 2.94	16.21ab ± 3.86	18.36ab ± 5.46			

Table 4.3.F: Total P content, P concentration, total N content, and N concentration in the belowground biomass in the RC of the split-root pot that received the higher amount of N. For further explanation, see Tab. 4.3.A.

N supply (mg kg-1)		150	180	210	255	Significances		
						AM fungal strain	N supply	Inter-action
P content in the root and tuber (mg per RC)	GM	5.13 ± 1.50	6.70 ±1.43	2.34 ± 1.84	3.26 ± 2.57	ns	ns	ns
	GI	5.53 ± 1.96	2.30 ± 2.03	2.93 ± 3.27	6.60 ± 6.35			
P concentration in the root and tuber (mg g^{-1} DW)	GM	1.10 ± 0.12	0.90 ± 0.14	1.18 ± 0.29	1.20 ±0.08	ns	ns	ns
	GI	1.41 ± 0.38	1.43 ± 0.43	1.13 ± 0.35	1.23 ± 0.46			
N content in the root and tuber (mg per RC)	GM	81.69ab ± 18.19	117.40b ± 14.95	46.12a ± 23.60	62.74ab ± 41.61	ns	ns	*
	GI	62.57a ± 28.14	41.31a ± 20.01	49.18a ± 29.03	92.50ab ± 63.76			
N concentration in the root and tuber (mg per g DW)	GM	21.63 ± 2.47	15.86 ± 1.47	25.98 ± 5.66	24.26 ± 5.73	ns	ns	ns
	GI	21.58 ± 5.05	29.98 ± 2.94	26.39 ± 6.26	25.71 ± 10.61			

Shoot N concentration of plants inoculated with *G. intraradices* tended to be higher compared with plants inoculated with *G. mosseae*, particularly in the N supply treatments (Tab. 4.3.D), but total N content of plants inoculated with *G. intraradices* and with *G. mosseae* was not significantly different (Tabs. 4.3.A and 4.3.D).

The P and N content in belowground biomass (root and tuber) of plants inoculated with either *G. mosseae* or *G. intraradices* was not significantly affected by either P or N supply treatments. There was no significant difference in P and N content in belowground biomass between plants inoculated with *G. mosseae* and with *G. intraradices* in either P or N supply treatments (Tabs. 4.3.B, 4.3.C, 4.3.E and 4.3.F).

However, P concentration in the belowground biomass of plants inoculated with either *G. mosseae* or *G. intraradices* tended to be decreased in the RC that received a lower amount of P (Tab. 4.3.C).Phosphorus concentration in the belowground biomass of plants inoculated with *G. intraradices* tended to be higher than that of plants inoculated with *G. mosseae* in either P or N supply treatments (Tabs. 4.3.B, 4.3.C, and 4.3.E).

Nitrogen concentration in belowground biomass tended to be decreased in the RC that received a lower amount of P. This applied to plants inoculated with either *G. mosseae* or *G. intraradices* (Tab. 4.3.B),and in the RC that received a lower amount of N particularly to

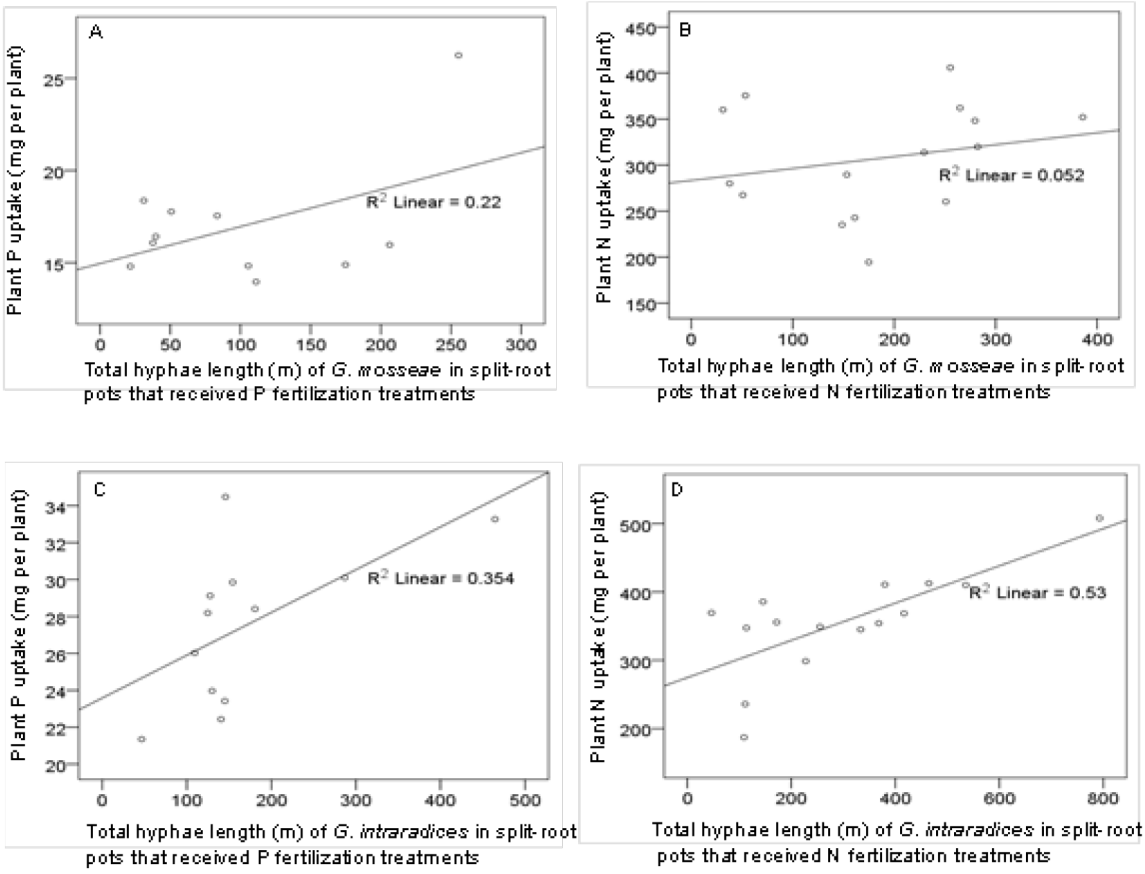

Figure 4.1: The relationship between the total hyphal length of either *G. mosseae* or *G. intraradices* in split-root pots that received either P or N fertilization treatments and plant P or N uptake, respectively.

plants inoculated with *G. mosseae* (Tab. 4.3.E). There was no significant difference between plants inoculated with *G. mosseae* and *G. intraradices* in N concentration of the belowground biomass (Tabs. 4.3.B, 4.3.C, 4.3.E, and 4.3.F).

4.4.4 THE RELATIONSHIP BETWEEN HYPHAE LENGTH AND PLANT PHOSPHORUS OR NITROGEN UPTAKE

The total hyphae length of *G. mosseae* in split-root pots that received either P or N fertilization treatments was not significantly correlated with the plant total P or N uptake (Fig. 4.1; statistics not shown). In contrast, in *G. intraradices* total hyphae length was positively correlated with the total plant P or N uptake. In particular, increasing hyphae length of *G. intraradices* was related to increased plant N uptake.

4.5 DISCUSSION

In the present experiment, sweet potato plants colonized by *G. intraradices* had higher plant P content, tissue P concentration and plant DW than corresponding plants colonized by *G. mosseae* in either P or N supply treatments. This indicated that *G. intraradices* was more effective than *G. mosseae* in promoting nutrient uptake and hence plant growth.The higher effectiveness of *G. intraradices* compared with *G. mosseae* to increase nutrient uptake might be caused by the higher extent of AM root colonization in *G. intraradices* than in *G. mosseae*. Conversely, *G. mosseae* was a faster root colonizer than *G. intraradices* in a study of Jansa et al. (2008).The extent of AM root colonization is often, but not always, positively correlated with AM contribution to plant performance (Pietikäinen et al., 2007). It has to be kept in mind though that the estimation of the extent of AM root colonization usually includes both living and dead fungal material, and thus any conclusion on current fungal activity has to be drawn with care. The present data also provide only semi-quantitative evidence of fungal development because colonization intensities (numbers of arbuscules and vesicles per colonized intersection) were not recorded.

The extent of AM colonization from both *G. mosseae* and *G. intraradices* in this study was relatively low, also compared to earlier studies with the same plant species in our group (see Chapter 3 of this thesis). This is very likely due to the fact that the inoculum of both fungi was applied as late at 49 d after planting. Amijee et al. (1993) suggested that, as root cell age, they may become progressively less susceptible to colonization by an AM fungus. Thus, there would be fewer entry points per unit root length when the older plants are inoculated, leading to a reduced overall rate of colonization compared with root systems that were in contact with AM mycelia from an early age on.

Nevertheless, the data of the present study clearly showed that the isolates of *G. mosseae* and *G. intraradices* used in this study exhibit different patterns of extraradical mycelia growth, as measured by the ratio of coarse to thin hyphae. *Glomus intraradices* apparently produced thin (absorbing) hyphae more than *G. mosseae* in either P or N supply treatments. The lower proportion of coarse (runner) than of thin (absorbing) hyphae in *G. intraradices* compared with *G. mosseae* may indicate a higher mycelial activity in P uptake (Olsson et al., 2006). However**,** the short life-span and rapid turnover of fine absorbing hyphae need to be considered.The differences in P or N acquisition of plants inoculated with *G. mosseae* were not correlated with the production of external hyphae (total hyphae length in the two HC). In contrast, hyphae length of *G. intraradices* was positively

correlated with plant P or N acquisition. It has to be kept in mind, however, that it cannot be completely excluded that AM fungal mycelia in the HC (in distance to host plants roots) might differ in their architecture and activity from mycelium produced in the RC in the vicinity of roots.

The hyphae lengths from the two fungi were not significantly different, even though the extent of AM colonization was significantly different between *G. mosseae* and *G. intraradices.* The extent of AM colonization is not always proportional to the size of the external mycelium(Dodd et al., 2000),but more often proportional to spore production. In the present study, the number of spores per unit mycelium DW was lower in *G. mosseae* than in *G. intraradices*, corresponding with the lower extent of AM colonization in *G. mosseae* than in *G. intraradices*.

With respect to AM fungal development in RC and HC, increasing concentration of P in one side RC of the two RC of the split-root system did not reduce the extent of AM fungal colonization from *G. intraradices*. Also in another experiment, high P concentration of soil did not inhibit root colonization by an AM fungus as long as the overall plant P nutritional status was low (Bücking and Shachar-Hill, 2005). In the present study, plant P and N status as indicated by shoot P and N concentration of sweet potato plants colonized by either *G. mosseae* or *G. intraradices* was indicative of deficiency following Munson (1998), and this was not affected by either P or N supply treatments. The belowground biomass P concentration of plants colonized by *G. intraradices* was also low and not affected by P supply treatments. The internal P status of plants, particularly root P concentration, very likely controlled the extent of AM colonization and sporulation of these fungi in our study (Olsson et al., 2006). The extent of AM colonization and number of spores (per m hyphae length and per weight of mycelium) of *G. intraradices* was not affected by P supply treatments because shoot and root P concentration of plants colonized by *G. intraradices* were not affected by P distribution in the soil.

However, different AM fungi show different responses to soil P concentrations. The level of AM fungal colonization of *G. mosseae* was decreased in the RC that received either a higher or a lower amount of P compared to plants with equal P distribution across both root halves. This effect was observed even though P concentrations in the shoot and in the belowground biomass of plants colonized by *G. mosseae* were still low and not affected by P supply treatments. The lower level of AM root colonization in the RC that received a higher amount of P might be caused by the increased root growth and hence a reduced ratio of colonized to uncolonized root length. This effect can occur independent of any effect of

higher soil P on plant suppression or control of fungal activity (Smith et al., 2011). On the other hand, the lower level of AM root colonization in the RC that received the lowest amount of P might be caused by competition between the plant host and the AM fungi for the scarce P (Peters and Habte, 2001).

Also the N concentration in the root tissue can affect root colonization (Bressan, 2001). Belowground biomass N concentration, particularly in plants colonized by *G. mosseae*, tended to be lower in the RC that received a lower amount of N. The extent of AM colonization from both isolates, however, was not affected by N supply treatments. In contrast, the number of spores per meter hyphae length from both isolates was reduced in the HC that received a lower amount of N. Nitrogen is required for spore formation because N is a principal component of chitin which is abundant in the spore wall (Bago et al., 2004).

The length of extraradical hyphae and the weight of the extraradical mycelium of *G. mosseae* or *G. intraradices* were also not affected by either P or N supply treatments. Both AM isolates did not decrease or increase hyphal length densities in the RC that received either lower or higher amount of either P or N, respectively. This indicates that these nutrients were not locally limiting the growth of the AM fungi and that the AM fungi both did not specifically forage for areas of high mineral P or N concentration in soil. The activity of the fungal mycelium to forage for P can be shown by the ratio of coarse (runner) to thin (absorbing) hyphae (Olsson et al., 2006). This ratio in both *G. mosseae* and *G. intraradices* was not affected by either P or N supply treatments. The activity of fungal mycelium to forage for nutrients in the soil may also be regulated by the plant P status (Nagy et al., 2009).

In the present study, sweet potato plants showed a high ability for nutrient translocation within the plant and nutrient integration for shoot growth, confirming results reported in Chapter 3 of this thesis. This is reflected by biomass production and shoot dry weight of plants grown in hetero- and homogeneous either P or N supply treatments. Plants grown in heterogeneous either P or N distribution had an equal biomass compared to plants grown in homogeneous either P or N distribution when the same quantity of nutrients was supplied. Shoot P and N concentration of plants colonized by either *G. mosseae* or *G. intraradices* were also not affected by either P or N supply treatments. Plant P or N status, particularly the shoot P and N concentration, controls on plant P and N uptake demands and root proliferation in a high nutrient rich patch (Lima et al., 2010; Ma and Rengel, 2008), so that total root dry weight of plants supplied with homogeneous and heterogeneous nutrient distribution was also not significantly different in the present and earlier experiments.

In the present study, shoot P and N concentrations of sweet potato plants were

indicative of deficiency when compared to standard values (Munson, 1998). Plants deficient in a certain nutrient may readily take up this nutrient from soil when available, irrespective of homogeneous and heterogeneous distribution of that nutrient in the soil. When the efficiency of root foraging in the nutrient rich patch is reduced because of nutrient depletion in the patch, there is no longer a difference in shoot biomass production between plants grown in soil with homogenous and heterogeneous nutrient distribution (Kembel and Cahill, 2005).

Plant P and/or N status regulate the dry matter allocation between shoot and root (Hammond and White, 2008; Paponov et al., 2000). Plant P and N status in the present study as shown by shoot P and N concentration were not affected by either P or N supply treatments. Both shoot/root and aboveground/belowground ratio of plants colonized by either *G. mosseae* or *G. intraradices* were not affected by either P or N supply treatments. In the present experiment, plants colonized by either *G. mosseae* or *G. intraradices* did not differ in their shoot/root ratio and aboveground/belowground ratio with either hetero- or homogeneous P and N distributions. Even though shoot P and N concentrations of plants colonized by *G. intraradices* were higher than that of plants colonized by *G. mosseae*, particularly in the 255:45 N distribution treatment, the shoot/root and aboveground/belowground ratio were not significantly different. It has to be kept in mind though that tuber formation was highly variable between treatments in the present experiment, so that statistical evidence is not very strong. Further experiments, perhaps using larger pots and a longer experimental time, are required to study mycorrhizal effects and influences of local nutrient supply on tuber formation.

The ratio of root dry weight of the two halves of the root system of plants colonized by either *G. mosseae* or *G. intraradices* was affected by either P or N supply.Root dry weight in the present experiment tended to be higher in RC that received a higher amount of either P or N. Roots often proliferate in nutrient-rich patches when they encounter the patches (Hodge, 2004). Mycorrhizal roots may have a modified response from non-mycorrhizal roots when they encounter P patches (see Chapter 3 of this thesis). However, in the present experiment the inoculum of both fungi was applied late at 49 days after planting. Root dry weight of mycorrhizal plants tended to be higher in the RC that received a lower amount of P when the AM fungus inoculum was applied in the beginning of plant growth (see Chapter 3). In contrast, under these conditions non-mycorrhizal and mycorrhizal roots proliferated in N rich patches (see also Chapter 3). Roots do not require mycorrhizal assistance to capture inorganic N because inorganic N (in particularly NO_3^-) readily moves to the roots via diffusion (Hodge and Fitter, 2010).

The ratio of belowground DW of the two halves of the root system of plants colonized by either *G. mosseae* or *G. intraradices* was not affected by P supply treatments. N supply treatments affected the ratio of belowground DW of the two halves of the root system of plants colonized by *G. mosseae*, particularly in the 180:120 N distribution. The increase of this ratio was caused by an increasing root and tuber dry weight in the RC that received a higher amount of N.

From the present results it can be concluded that sweet potato plants respond to P or N rich soil sites by root proliferation, and that this helps to support similar plant P and N uptake under homogeneous and heterogeneous soil P and N supply. Extraradical AM mycelium from *G. mosseae* and *G. intraradices* did not actively forage for P or N rich patches. Arbuscular mycorrhizal fungi may rather increase plant nutrient uptake by altering the capacity of roots to take up nutrients in the nutrient rich patch (Gavito and Olsson, 2003). The extent of plant growth promotion by AM fungi depends on the plant and fungal genotype combination. In this study, *G. mosseae* was less effective in increasing nutrient uptake and growth of sweet potato plant than *G. intraradices*. This difference was related to differences in the extent of AM colonization and particularly in the development pattern of the extraradical mycelium.

5. EFFECTS OF COMPOST TYPE AND DISTRIBUTION ON PLANTS INOCULATED AND UNINOCULATED BY AN ARBUSCULAR MYCORRHIZAL FUNGUS GROWN IN SOIL OR PEAT SUBSTRATE

5.1 ABSTRACT

Application of compost can serve as an alternative practice to mineral fertilizer use. The type, the quality and the placement of compost in soil must all be regarded in their effect on plant growth. Often, composts have low concentration of plant available nutrients. A high rate of compost applicationhowever, is ofteninsufficient to deliver adequate amounts of plant available nutrients, because the nutrients released from the compost may not be used effectively by the plants. In consequence, they will contaminate water and soil. Arbuscular mycorrhizal (AM) may be relevant in this respect, because the mycorrhizal symbiosis can make a contribution to increase plant uptake of P and other nutrients with limited availability in soil. The objective of this study was to assess the effects of mineral fertilizer or compost amendments to mineral soil or peat-based substrate and of an AM fungus inoculant. Marigold plants (*Tagetes patula*) inoculated as well as uninoculated with an AM fungal isolate of *Glomus mosseae* were grown in either soil or peat substrate. They were supplied with compost distributed homogeneously, in pellet form, and in a layer, with mineral fertilizer, and with fresh or dead compost tea. The results showed that mineral fertilization in the cultivation of marigold plants could be replaced by application of compost. However, the type of compost should be considered depending on the type of growth substrate. Application of solid compost gave more benefits to plant growth and flowering, when it was applied to soil substrate rather than to peat substrate. Conversely, application of compost tea was more beneficial to plant growth and flowering, when the compost tea was applied to peat substrate rather than to soil substrate. *Glomus mosseae* did not give a positive response to plant growth and flowering in both substrates, when compost or compost tea was applied. Tests to determine which AM inoculants perform best in different growth substrates should be conducted to obtain a synergism between AM fungal isolate, type of fertilizer and of growth substrate, so that a benefit from using biological and organic fertilizers at the same time can be achieved.

5.2 INTRODUCTION

Massive applications of mineral fertilizer have a direct negative impact on the physical, chemical and biological properties of the soil, and increase the risk of degradation of soil (Tejada and Gonzales, 2006). In addition, for most small-scale farmers mineral fertilizers are expensive (Inckel et al., 2005, p.8). Organic material (fertilizers) such as compost can serve as an alternative to mineral fertilizer (Golabi et al., 2006). Compost is the product of a controlled aerobic decomposition of organic matter, resulting in a stable, dark

brown, soil-like material (Rouse et al., 2008, p. 17). Beside the nutrients released supply with the organic material, compost has beneficial effects on soil structure and soil biota (Perner et al., 2006). This is due to its high content of organic matter (Rivero et al., 2004) and also due to the richness in microorganisms that help plant to mobilize and acquire nutrients (Ghehsareh et al., 2011).

The use of a compost extract, the so-called 'compost tea', is gaining popularity in organic agriculture (Hargreaves et al., 2009). Applications of compost tea to soil have two main aims: to add nutrients, and to inoculate microbial life to the soil. This application may potentially benefit plant growth through a direct nutritional benefit, or by increased mineralization, or by disease protection from soil-borne pathogens (Shrestha et al., 2012).

In earlier studies, the effects of compost on plant growth and yield were found to vary, dependingon compost type (solid or liquid) (Scheuerell, 2004), quality (Fuchs et al., 2008) and placement in the soil (Baiyeri and Tenkouano, 2008; Melo et al., 2012) as well as on soil type (Doesken, et al., 2007). Application of compost tea has very little effect on the physical properties of the growth substrate compared to compost application (Scheuerell, 2004). Concerning the compost placement in soil, Csizinszky and Stanley (1998) and Khalilian et al. (2000) reported that there were no differences between banded and broadcasted compost on tomato yield, and between subsurface and surface municipal solid waste placement on cotton yield. However, Baiyeri and Tenkouano (2008) reported that plantain hybrids supplied with manure placed on the soil surface had highest plant height and total leaf area compared to plants supplied with manure either placed below the soil surface, or a treatment with 50% manure placed at the soil surface and 50% at below the soil surface. In addition to placement effects, the mineralization rate of composts is also influenced by the type of soil where the compost is applied (Doesken et al., 2007).

Concentrations of plant available nutrients in the compost are usually low (Hogarh et al., 2008) and are not sufficient to promote plant growth (Hüttl and Fussy, 2001). The low nutrient concentration in compost is caused by its slow mineralization rate (Zwart, 2001). Therefore, it is often held necessary to accompany compost application with a mineral fertilizer amendment to support adequate plant growth (Hüttl and Fussy, 2001). On the other hand, the use of mineral fertilizer is not always possible and there is a need to reduce mineral fertilizer application (Myint et al., 2010). A high rate of compost application is often not indicated because the nutrients that are not used effectively by the plants may later contaminate water and soil (Yusuff et al., 2007).

Arbuscular mycorrhizal (AM) fungi may be very relevant in this situation. This symbiosis makes a contribution to increased plant absorption of P and other elements with limited availability in soil (Cornejo et al., 2008). In an earlier study, however, application of a compost and a mycorrhizal fungus together did not result in a synergism to increase plant nutrient uptake and hence plant growth (Perner et al., 2006). The outcome may depend on the type of compost material. For example, composted agricultural plant waste, cow manure and wheat straw had positive effects on AM root colonization (Valarini et al., 2009) while composted beef feedlot manure reduced AM root colonization (Garcia et al., 2007).

In commercial crop production, peat-based substrates are often used as plant growth substrate instead of soil. In conventional production systems, this substrate is usually supplemented with mineral fertilizer to achieve optimal nutrient supply for plant growth (Perner et al., 2006). As plant growth substrate, peat has lower bulk density, provides better aeration, and has a higher water-holding capacity than mineral soil. Plant growth often benefits from these specific rhizosphere conditions (Corkidi et al., 2004). Peat effects on mycorrhizal colonization are not consistent. Peat can have positive (Matysiak and Falkowski, 2010) or negative (Vestberg and Kukkonen, 2008) effects on AM root colonization. Linderman and Davis (2003) reported that increased or decreased AM root colonization due to peat amendment was depended on the type of mycorrhizal fungus used.

However, in general terms peat substrate is usually less favorable for the development of mycorrhizal colonization than growth substrates containing soil (Corkidi et al., 2004). The negative effect of peat substrate towards mycorrhization is related to its low P buffer capacity (Peters and Habte, 2001) and its specific rhizosphere conditions (Corkidi et al., 2004). By controlling P availability in peat, the function of AM fungi could be similar to mineral soil (Estaún et al., 2003). Compost application can significantly increase P concentration in a peat-based substrate (Zhang et al., 2004).

Regardless of the composted material or substrate, compost acts as a slow release fertilizer (Hunter et al., 2012; Kraus and Warren, 2000) which is resulting in a long-term plant availability of P (Syers et al., 2008, p.46). The placement of nutrients must also be considered to achieve rapid nutrient uptake of plants (Baiyeri and Tenkouano, 2008), even though the external hyphae of arbuscular mycorrhizal can increase the surface area of plants for nutrient uptake (Neumann and George, 2009).

The objectives of the current study were to assess the effects of mineral and organic fertilizer amendment to mineral soil and to peat on the infectivity of a mycorrhizal inoculant. I also analyzed plant growth response to this inoculant in the different substrates amended by

either mineral or organic fertilizer. I hypothesized that application of an appropriate AM fungus together with compost can replace mineral fertilizer utilization in agricultural or horticultural practice and that difference in the compost distribution in the growth substrate will also affect plant nutrient uptake and plant growth.

5.3 MATERIALS AND METHODS

5.3.1 EXPERIMENTAL PLANT PREPARATION

Marigold plant was chosen as the experimental plant. The marigold cultivar used in this study was *Tagetes patula* 'Mr. Majestic'. Selected marigold seeds were surface sterilized in sodium hypochlorite (50 ml per liter) for 30 seconds and germinated on filter paper soaked with saturated $CaSO_4$ solution. Seedlings were maintained in beaker glasses covered with aluminum foil and were watered as required to avoid drying of the filter paper. After seven days, the seedlings had developed one set of true leaves and some roots, and were individually transplanted into plastic pots.

5.3.2 PREPARATION OF THE PLANTING POTS AND MINERAL FERTILIZATION

Marigold plants were grown in 700 ml plastic pots that contained either sterilized loess soil ("soil", a loess substrate from a C horizon of a soil in Weihenstephan, Germany) or commercial growth substrate ("peat substrate"; Terreau Professionnel GePAC, Einheitserde, Germany). The sieved (4 mm) C-loess soil was sterilized by heating in the oven for 48 h at 80ºC to eliminate AM fungal propagules. Before heating, the soil contained (mg kg^{-1}): 5.2 and 3.4 $CaCl_2$ (0.0125 M) extractable NH_4^+, and NO_3^-, respectively; 4.4 acetate-extractable (CAL, Schuller, 1969) P; 58 CAL-extractable K; and 1.9 (Fe), 1.75 (Mn), 0.10 (Zn) and 0.16 (Cu) DPTA-extractable micronutrients. The soil had pH (0.01 M $CaCl_2$) of 7.3 and 0.2% organic matter. It was classified as loamy sand (45.5% sand, 42.0% silt, 12.8% clay) (Neumann and George, 2005). The peat substrate is a mixture blend of weakly decomposed white sphagnum peat, clay and other additives, containing per information supplied by the producer 1.5 g L^{-1} KCl, 150 mg L^{-1} N ($CaCl_2$ extraction), 150 mg L^{-1} P_2O_5 (CAL extraction) and 210 mg L^{-1} K_2O (CAL extraction). The peat substrate had a pH ($CaCl_2$) 5.8 and 75% organic matter.

In the treatments with mineral fertilizer application, both C-loess soil and peat substrate were fertilized with 200 mg N (NH_4NO_3), 60 mg P (KH_2PO_4), 200 mg K (K_2SO_4 +

KH_2PO_4), 100 mg Mg ($MgSO_4$) 10 mg Fe (FeEDTA), 10 mg Zn ($ZnSO_4.7H_2O$) and 10 mg Cu ($CuSO_4.5H_2O$). In the treatments with compost or compost tea application, both C-loess soil and peat substrate were fertilized per kg substrate with 20 mg P (KH_2PO_4), 40 mg Mg ($MgSO_4$), 10 mg Fe (FeEDTA), 10 mg Zn ($ZnSO_4.7H_2O$), 10 mg Cu ($CuSO_4.5H_2O$). Thecompost was commercial compost from Lumbrico Wumfarm, Germany, and contained according to the manufacturer 7,3 mg Al, 5163 mg Ca, 6.5 mg Fe, 1784 mg K, 1214 mg Mg, 5,8 mg Mn, 281 mg Na, 819 mg P, 84.5 mg S, 1.8 mg Zn per kg dry compost. The compost had pH (KCl) of 6.45, and a C concentration of 14.7%, an N concentration of 0.885% and a C/N ratio of 17. Single pots were filled with either 666 gram of a mixture of dry C loess soil and inoculum with a bulk density of 1.3 g cm^{-3} or 270 gram of a mixture of dry peat substrate and inoculum with a bulk density of 0.49 g cm^{-3}.

5.3.3 SET-UP OF THE INOCULATION AND OF THE COMPOST SUPPLY

In this experiment, for the mycorrhizal treatment (+M) each pot was inoculated with 30 gram inoculum of *Glomus mosseae* (mixture between *Glomus mosseae* BEG 12 and *Glomus mosseae* BEG 167 in the ratio 2:1). The inoculum was obtained from pot cultures of the respective AM fungi with maize plants on the same C loess soil, and consisted of air-dried soil with extraradical mycelium, AM spores and colonized root fragments. The inoculum was mixed homogeneously with the growth substrates before it was filled into the pot. For the non- mycorrhizal treatment (-M), the pot was inoculated with sterilized inoculum and 31 ml of an aqueous filtrate of inoculum, filtered through filter paper (VWR international no. 313 paper) to encourage a micoflora similar to that in the mycorrhizal treatment. Sterilized inoculum was obtained by heating in the oven 100ºC overnight.

There were two types of compost applications, i.e. raw compost (compost) and liquid composts (compost tea). For application of raw compost, per pot 92.7 gram of moist compost was added. There were three different modes of raw compost placement in the pots (distribution). Compost was either distributed homogenously in the substrate, or placed in a layer (1.5 cm from upper surface), or applied in pellet form. For the pellet treatment, three pellets were placed: one in the bottom of the pot, one in the middle, and one directly under the surface of the growth substrate. Application of compost tea consisted of fresh and dead compost tea. Dead compost tea was autoclaved compost tea. Pots were drenched with 35 ml either fresh compost tea or dead compost tea at 12 and 23 days after planting. The compost tea was made from 500 ml compost, 2000 ml deionized water and sugar mixed and aerated for two days, and filtered by 50 µm sieve plate. Phosphorus concentration in 1 ml compost tea

was 8.93 mM. Thus, in total there were six fertilizer application treatments (compost distributed homogeneously, compost distributed in a layer, compost distributed in pellet form, mineral fertilizer, fresh compost tea, dead compost tea), two substrates (soil and peat) and two levels of mycorrhizal inoculation (with and without).

5.3.4 PLANT GROWTH CONDITIONS

The pots were set up completely randomized in a greenhouse in Grossbeeren (long. 13º20'E; lat. 51º22'N), Germany for nine weeks from 12 May 2008 to 5 July 2008 with a light period of 16 h day/8 h night. Average light intensity was 709 µmol m^{-2} s^{-1} during the day, and there was not additional artificial light supply. Average air temperatures in the glasshouse during this time were 27ºC day/21ºC night, and relative humidity averaged 50%. All planting pots of this experiment changed their position on the planting table at regular intervals but a completely randomized design was maintained. The gravimetric water content of the substrate was adjusted to approximately 17% w/w in C loess soil substrate and 55% w/w in peat substrate after the plants was inserted. Water loss from the pot was estimated gravimetrically, and was replaced by deionized water every two days in the beginning and every day in the last week before harvest.

5.3.5 HARVEST AND ANALYSIS OF PLANTS AND AM FUNGAL MATERIAL

One week before harvest, the number of flowers and open flowers were counted. The counts of number of flowers was based on bud flower until blossom flower stage, while the counts of numbers of open flower was based on the opened corolla of the flower. At the time of harvest, shoots were cut off, and the roots were washed from the soil or peat of each pot with tap water. Shoots and roots dry weights were measured with a balance after drying at 80ºC for 48 h in the oven.

To assess the AM fungal colonized roots length, a representative sample of fresh roots was taken from each plant. The root samples were cleared and stained with trypan blue in lactic acid according to Philip and Hayman (1970). Approximately 100-200 root intersections were counted for mycorrhizal colonization by a gridline intersection procedure according to Giovannetti and Mosse (1980). The extent of AM fungal root colonization was expressed as the AM fungal colonized root length in percent of the total root length.

Dried shoot and belowground biomass from each plant was ground into fine powder. Shoots were ground in a rotation mill (ZM 100, Retsch, Germany) to the size of 0.25 mm and roots were ground in a Fritsch Pulverisette mill. Approximately 200 mg of ground plant

material were digested for 20 min in Teflon vessels in a microwave, together with 5 ml of 60% HNO_3 and 2 ml 30% H_2O_2. The solution was taken up into 25 ml of distilled water and filtered through blue ribbon filter paper (Rundfilter Macherey-Nagel 616/125 mm). Phosphate concentration in the liquid samples were measured photometrically (EPOS Analyzer 5060) after addition of molybdate-vanadate solution (Gericke and Kurmies, 1952). The total P uptake of plants was calculated by multiplying their biomass with their P concentration (for roots and shoots separately).

The quantitative extraction of N from plant material was done by explosive combustion in an oxygen enriched helium atmosphere surrounded by a copper oxide filled pipe at a temperature of 980ºC (Elementar Vario EL). The resulting gas mix was submitted to a gas-phase chromatograph where C and N could be quantified in a thermal conductivity tube. The total N uptake of plants was calculated by multiplying their biomass by their N concentration (for roots and shoots separately).

Unfortunately, the total P and N content and P and N concentrations of the plants grown in soil substrate fertilized by mineral fertilizer or compost teas could not be determined. The biomass of these plants was small and not sufficient for nutrient analysis with the methods available to me at the IGZ.

5.3.6 STATISTICAL ANALYSIS

The experiment was a completely randomized design with four replicates per treatment. Treatment effects were statistically analyzed by SPSS (SPSS 15, SPSS Inc. Chicago, USA). A Two-Way ANOVA was conducted to assess whether fertilization treatments and the growth substrate had a significant effect on the mean values. In addition, mean values obtained for non-mycorrhizal and mycorrhizal plants within the same growth substrate and fertilization treatment were compared by t-tests. Alternatively, when the growth substrate was not considered in the comparison, a Two-Way ANOVA was conducted to assess whether fertilizer and AM fungal treatments had a significant effect on the mean values. A Duncan Multiple Range Test was conducted to identify significant difference between the mean values. In all tests, differences were considered significant when $P<0.05$.

5.4 RESULTS

5.4.1 PLANT DRY WEIGHT AND SHOOT/ROOT RATIO

Non-mycorrhizal and mycorrhizal plants grown in peat substrate had higher shoot and total plant DW compared to non-mycorrhizal and mycorrhizal plants grown in soil substrate. This was particularly the case when both substrates were fertilized with mineral fertilizer or compost teas (Tab.5.1.A).

Table 5.1.A: Total plant and shoot DW of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate fertilized with compost, mineral fertilizer or compost tea.

Fertilizer	Growth substrate	Total plant DW		Shoot DW	
		-M	+M	-M	+M
Compost distributed homogeneously	Soil	6.14 ± 0.50c	4.97 ± 0.32b●	5.06 ± 0.33d	4.24 ± 0.28bc●
	Peat	6.40 ± 0.62c	6.19 ± 0.59c	4.91 ± 0.47d	4.61 ± 0.45bc
Compost distributed in pellet form	Soil	6.14 ± 0.66c	5.17 ± 1.76b	4.97 ± 0.50d	4.27 ± 1.01bc
	Peat	4.53 ± 0.89b	5.73 ± 0.35bc	3.43 ± 0.65b	4.10 ± 0.15b
Compost distributed in a layer	Soil	5.43 ± 0.89bc	6.16 ± 0.41c	4.29 ± 0.60cd	4.81 ± 0.25c
	Peat	5.93 ± 0.36c	6.45 ± 0.75c	4.45 ± 0.29cd	4.79 ± 0.48c
Mineral fertilizer	Soil	0.28 ± 0.16a	0.19 ± 0.02a	0.24 ± 0.13a	0.16 ± 0.02a
	Peat	4.78 ± 1.50b	7.59 ± 0.21d●	3.85 ± 0.96bc	6.02 ± 0.10d●
Fresh compost tea	Soil	0.49 ± 0.42a	0.11 ± 0.05a	0.40 ± 0.32a	0.08 ± 0.03a
	Peat	8.59 ± 0.36d	8.03 ± 0.47d	6.76 ± 0.05e	6.27 ± 0.33d
Dead compost tea	Soil	0.79 ± 0.38a	0.14 ± 0.11a●	0.67 ± 0.29a	0.08 ± 0.03a●
	Peat	8.22 ± 0.59d	7.65 ± 0.22d	6.51 ± 0.38e	5.95 ± 0.25d
Statistical significance	Growth substrate	*	*	*	*
	Fertilizer	*	*	*	*
	Interaction	*	*	*	*

Values are means and SD. Mean values followed by the same letter in the same column are not significantly ($P<0.05$) different. The mean value obtained for a mycorrhizal treatment (+M) followed by a black dot is significantly ($P<0.05$) different from the mean value obtained for the corresponding non-mycorrhizal treatment (-M).

Table 5.1.B: Root DW and shoot/root ratio of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate fertilized with compost, mineral fertilizer or compost tea. For explanation, see Table 5.1.A.

Fertilizer	Growth substrate	Root DW		Shoot/root ratio	
		-M	+M	-M	+M
Compost distributed homogeneously	Soil	0.98 ± 0.20bc	0.66 ± 0.07b●	4.78 ± 0.78abc	5.79 ± 0.59c
	Peat	1.40 ± 0.22cd	1.49 ± 0.20de	3.30 ± 0.31a	2.94 ± 0.28ab
Compost distributed in pellet form	Soil	0.98 ± 0.30bc	0.74 ± 0.28b	4.45 ± 1.05ab	4.93 ± 0.95cd
	Peat	0.98 ± 0.29bc	1.30 ± 0.17cd	3.18 ± 0.58a	2.53 ± 0.27a
Compost distributed in a layer	Soil	0.91 ± 0.36bc	1.15 ± 0.21c	3.97 ± 0.88ab	3.61 ± 0.54abc
	Peat	1.40 ± 0.21cd	1.59 ± 0.41de	3.04 ± 0.42a	2.98 ± 0.63ab
Mineral fertilizer	Soil	0.02 ± 0.01a	0.02 ± 0.01a	6.75 ± 1.41bc	7.95 ± 2.30d
	Peat	0.87 ± 0.54b	1.49 ± 0.15d●	5.04 ± 2.20abc	3.86 ± 0.34abc
Fresh compost tea	Soil	0.04 ± 0.05a	0.01 ± 0.01a	5.73 ± 1.43bc	4.47 ± 1.58bcd
	Peat	1.76 ± 0.38d	1.68 ± 0.24e	3.80 ± 0.75ab	3.62 ± 0.49abc
Dead compost tea	Soil	0.06 ± 0.05a	0.03 ± 0.04a	6.86 ± 2.76c	3.04 ± 2.00ab
	Peat	1.62 ± 0.37d	1.62 ± 0.11de	3.89 ± 0.69ab	3.52 ± 3.32abc
Statistical significance	Growth substrate	*	*	*	*
	Fertilizer	*	*	*	*
	Interaction	*	*	ns	*

Shoot and total DW of non-mycorrhizal and mycorrhizal plants tended to be less affected by the type of growth substrate when both substrates were fertilized with compost.

In the soil substrate, non-mycorrhizal and mycorrhizal plants had higher shoot and total plant DW when this substrate was fertilized with compost than when it was fertilized with mineral fertilizer or compost teas (Tab. 5.1.A). On the contrary, in peat substrate non-mycorrhizal and mycorrhizal plants had a higher plant DW when this substrate was fertilized with mineral fertilizer or compost teas than when it was fertilized with compost. There was no significant difference between compost treatments distributed homogeneously, in pellet form or in a layer on shoot DW of non-mycorrhizal and mycorrhizal plants grown in soil substrate.

There was no significant difference between application of mineral fertilizer and compost teas on shoot and total DW of non-mycorrhizal and mycorrhizal plants grown in soil substrate. Shoot and total DW of non-mycorrhizal plants was higher when peat substrate was fertilized with compost teas than when it was fertilized with mineral fertilizer; however, total and shoot DW of mycorrhizal plants was not significantly different in this case. Application

of fresh and dead compost tea was not significantly different in its effect on total DW of non-mycorrhizal and mycorrhizal plant in both substrates.

Application of AM fungi did not increase shoot and plant DW when both substrates were fertilized with either compost or compost teas. In fact, plant DW showed a negative response to AM colonization particularly when soil substrate was fertilized with compost distributed homogeneously or with dead compost tea. However, plant DW showed a positive response to AM colonization when the peat substrate was fertilized with mineral fertilizer only.

Mycorrhizal and non-mycorrhizal plants grown in peat substrate had higher root DW compared to mycorrhizal and non-mycorrhizal plants grown in soil substrate, particularly when both substrates were fertilized with mineral fertilizer or compost teas (Tab. 5.1.B). There was no significant difference between soil and peat substrate on root DW of non-mycorrhizal plants when both substrates were fertilized with compost. However, root DW of mycorrhizal plants grown in peat substrate was higher than root DW of non-mycorrhizal plants grown in soil substrate in all fertilization treatments.

In soil substrate, non-mycorrhizal and mycorrhizal plants had higher root DW when this substrate was fertilized with compost than when it was fertilized with mineral fertilizer or compost teas (Tab. 5.1.B). In contrast, in peat substrate there was no significant difference between application of compost, mineral fertilizer and compost teas on root DW of non-mycorrhizal and mycorrhizal plants.

There was no significant difference between compost distributed homogeneously, in pellet form and in a layer on root DW of non-mycorrhizal plants grown in either substrate. However, in soil substrate mycorrhizal plants had higher root DW when this substrate was fertilized with compost distributed in a layer than when it was fertilized with compost distributed homogeneously or in pellet form.

There was no significant difference between application of mineral fertilizer and compost teas on root DW of non-mycorrhizal and mycorrhizal plants grown in soil substrate (Fig. 5.1.B). In contrast, in peat substrate root DW of non-mycorrhizal and mycorrhizal plants was higher when this substrate was fertilized with compost teas than when it was fertilized with mineral fertilizer. Application of fresh and dead compost tea was not significantly different in the effect on root DW of non-mycorrhizal and mycorrhizal plants in both substrates. Application of AM fungi increase root DW when peat substrate was fertilized with mineral fertilizer.

There was no significant difference between soil and peat substrate in the effect on shoot/root ratio of non-mycorrhizal plants when both substrates were fertilized with compost (Tab. 5.1.B). However, shoot/root ratio of mycorrhizal plants was higher in soil substrate than in peat substrate when compost was amended.

In the soil substrate, shoot/root ratio of non-mycorrhizal plants tended to be higher when this substrate was fertilized with mineral fertilizer or compost teas than when it was fertilized with compost. There was no significance difference between application of compost, mineral fertilizer and compost teas on shoot/root ratio of non-mycorrhizal plants grown in either soil or peat substrate. Application of fresh and dead compost tea was not significantly different in the effect on shoot/root ratio of non-mycorrhizal and mycorrhizal plants grown in either substrate.

Mycorrhizal plants had the highest shoot/root ratio when the soil substrate was fertilized with mineral fertilizer. There was no significant difference between application of compost, mineral fertilizer and compost teas on shoot/root ratio of non-mycorrhizal and mycorrhizal plants grown in peat substrate. Inoculation with AM fungi did not increase the shoot/root ratio on both substrates and with all types of fertilizers (Tab. 5.1.B).

5.4.2. THE NUMBER OF FLOWERS AND OPEN FLOWERS

There was no significant difference between soil and peat substrate in the effect on the number of flowers of non-mycorrhizal and mycorrhizal plants when both substrates were fertilized with compost (Tab. 5.2). However, non-mycorrhizal and mycorrhizal plants grown in peat substrate had a higher number of flowers than those grown in soil substrate when both substrates were fertilized with mineral fertilizer or compost teas. In soil substrate, there was no significant difference between applications of compost, mineral fertilizer, and compost teas in the number of flowers of non-mycorrhizal plants. The variation between replications for the number of flowers was high, so that standard deviations of means were also large.

The number of flowers tended to be higher in non-mycorrhizal and mycorrhizal plants grown in soil substrate fertilized with compost than when it was fertilized with mineral fertilizer or compost teas (Tab. 5.2). There was no significant difference between application of compost, mineral fertilizer and compost teas in their effect on number of flowers of mycorrhizal plants grown in peat substrate. There was no significant difference between compost distributed homogeneously, in pellet form or in layer on number of flowers of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate. There was also no significant difference between fresh and dead compost tea in their effect on the number of

flowers of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate. The number of flowers was increased by AM colonization when plants were grown in peat substrate fertilized with mineral fertilizer.

The number of open flowers of non-mycorrhizal plants grown in peat substrate tended to be higher than those of corresponding plants grown in soil substrate (Tab. 5.2). There was no significant difference between application of compost, mineral fertilizer and compost teas on the number of open flowers of non-mycorrhizal plants.

Table 5.2: The number of flowers and number of open flower of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate fertilized with compost, mineral fertilizer or compost tea.

Fertilizer	Growth substrate	Number of flowers		Number of open flowers	
		-M	+M	-M	+M
Compost distributed homogeneously	Soil	4.00 ± 2.16ab	6.50 ± 5.45ab	1.00 ± 0.00a	1.75 ± 1.50b
	Peat	5.25 ± 2.87ab	6.25 ± 2.22ab	1.50 ± 0.58ab	1.50 ± 0.58b
Compost distributed in pellet form	Soil	5.25 ± 3.20ab	4.75 ± 3.59ab	1.25 ± 0.50ab	1.25 ± 0.50b
	Peat	3.67 ± 1.53ab	4.75 ± 3.59ab	1.00 ± 0.00a	1.25 ± 0.50b
Compost distributed in a layer	Soil	2.75 ± 1.71ab	7.25 ± 4.57b	0.75 ± 0.50a	1.50 ± 0.58b
	Peat	7.00 ± 3.00bc	4.50 ± 1.91ab	1.33 ± 0.58ab	1.25 ± 0.50b
Mineral fertilizer	Soil	1.00 ± 0.82a	1.50 ± 0.71a	0.25 ± 0.50a	0.00 ± 0.00a
	Peat	3.00 ± 1.63ab	9.00 ± 3.65b●	0.25 ± 0.50a	1.00 ± 0.00b●
Fresh compost tea	Soil	1.75 ± 0.96a	1.25 ± 0.50a	0.25 ± 0.50a	0.00 ± 0.00a
	Peat	11.33 ± 5.51cd	7.50 ± 4.43b	1.67 ± 0.58ab	1.00 ± 0.00b
Dead compost tea	Soil	3.67 ± 2.52ab	1.00 ± 0.00a	0.67 ± 0.58a	0.00 ± 0.00a
	Peat	12.75 ± 5.38d	8.50 ± 2.65b	2.50 ± 2.38b	1.50 ± 0.58b
Statistical significance	Growth substrate	*	*	*	*
	Fertilizer	*	ns	ns	*
	Interaction	*	ns	ns	*

Values are means and SD. Mean values followed by the same letter in the same column are not significantly ($P<0.05$) different. The mean value obtained for a mycorrhizal treatment (+M) followed by a black dot is significantly ($P<0.05$) different from the mean value obtained for the corresponding non-mycorrhizal treatment (-M).

There was also no significant difference between soil and peat substrate in their effect on the number of open flowers of mycorrhizal plants when both substrates were fertilized with compost. However, mycorrhizal plants grown in peat substrate had a higher number of open flowers than those grown in soil substrate when both substrates were fertilized with mineral fertilizer or compost teas. There was no significant difference between compost distributed homogeneously, compost in pellet form and compost placed in a layer on number

of open flowers of mycorrhizal plants grown in either soil or peat substrate. Inoculation with AM fungi increased the number of open flowers particularly when plants grown in peat substrate were fertilized with mineral fertilizer.

5.4.3. THE RATE OF AM ROOT COLONIZATION

Non-inoculated plants were free of mycorrhiza. Inoculated plants grown in soil substrate tended to have a higher rate of AM root colonization than those grown in peat substrate (Tab. 5.3). The rate of AM root colonization was highest in plants grown in soil substrate when this substrate was fertilized with mineral fertilizer.

There was no significant difference between compost distributed homogeneously, in pellet form or in a layer on the rate of AM root colonization of plants grown in either soil or peat substrate. The rate of AM colonization of plants grown in soil substrate was higher when this substrate was fertilized with dead compost teas than when it was fertilized with fresh compost tea. In contrast, there was no significant difference between fresh and dead compost tea in the rate of AM root colonization of plants grown in peat substrate.

Table 5.3: The rate of AM root colonization of plants grown in either soil or peat substrate fertilized with compost, mineral fertilizer or compost tea.

Fertilizer		Growth substrate	
		Soil	Peat
Compost distributed homogeneously		4.45 ± 1.63a	4.94 ± 2.70a
Compos distributed in pellet form		10.48 ± 4.19ab	6.58 ± 3.43a
Compost distributed in a layer		8.87 ± 3.65ab	4.69 ± 1.84a
Mineral fertilizer		26.48 ± 10.97d	16.08 ± 1.09bc
Fresh compost tea		8.18 ± 5.93ab	7.86 ± 3.88ab
Dead compost tea		19.55 ± 11.78cd	6.25 ± 3.78a
Statistical significance	Growth substrate	*	
	Fertilizer	*	
	Interaction	ns	

Values are means and SD. Mean values followed by the same letter are not significantly (P<0.05) different.

5.4.4. PLANT TOTAL PHOSPHORUS AND NITROGEN CONTENT

Non-mycorrhizal and mycorrhizal plants grown in peat substrate had higher plant P content than those grown in soil substrate (Tab. 5.4.A). In soil substrate fertilized with

compost, total P content of non-mycorrhizal and mycorrhizal plants was higher when compost was distributed in a layer than when it was distributed homogeneously or in pellet form. In peat substrate fertilized with compost, plant P content of non-mycorrhizal and mycorrhizal plants was lowest when compost was distributed in pellet form.

In peat substrate, non-mycorrhizal and mycorrhizal plants fertilized with compost or compost teas had a higher total P content than those fertilized with mineral fertilizer (Tab. 5.4.B). Non-mycorrhizal and mycorrhizal plants had a higher total P content when peat substrate was fertilized with compost distributed homogeneously or in a layer compared to the treatment where the compost was distributed in pellet form.

Table 5.4.A: Plant total P and N content of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate fertilized with compost.

Fertilizer	Growth substrate	Plant P content		Plant N content	
		-M	+M	-M	+M
Compost distributed homogeneously	Soil	9.42 ± 0.53ab	7.57± 0.44a●	56.05 ± 2.44c	51.72 ± 1.61c●
	Peat	11.86 ± 0.65c	11.61 ± 0.28c	50.15 ± 0.91b	46.84 ± 2.34b
Compost distributed in pellet form	Soil	8.33 ± 0.62a	7.83 ± 1.39a	58.45 ± 0.98c	60.55 ± 0.96d●
	Peat	9.50 ± 1.34ab	9.65 ± 0.65b	39.56 ± 4.30a	42.98 ± 1.87a
Compost distributed in a layer	Soil	10.00 ± 0.93b	9.98 ± 0.85b	56.40 ± 1.31c	57.48 ± 0.87c
	Peat	13.27 ± 1.50c	12.40 ± 0.29c	51.43 ± 2.26b	55.36 ± 1.70c●
Statistical significance	Growth substrate	*	*	*	*
	Fertilizer	*	*	*	*
	Interaction	ns	ns	*	*

Values are means and SD. Mean values followed by the same letter in the same column are not significantly ($P<0.05$) different. The mean value obtained for a mycorrhizal treatment (+M) followed by a black dot is significantly ($P<0.05$) different from the mean value obtained for the corresponding non-mycorrhizal treatment (-M).

Table 5.4.B: Plant total P and N content of non-mycorrhizal and mycorrhizal plants grown in peat substrate fertilized with compost, mineral fertilizer or compost tea. For explanation, see Table 5.4.A.

Fertilizer		Plant P content		Plant N content	
		-M	+M	-M	+M
Compost distributed homogeneously		11.86 ± 0.65cde	11.61 ± 0.28cde	50.15 ± 0.91bc	46.84 ± 2.34cd
Compost distributed in pellet form		9.50 ± 1.34ab	9.65 ± 0.65ab	39.56 ± 4.30a	42.98 ± 1.87ab
Compost distributed in a layer		13.27 ± 1.50e	12.40 ± 0.29de	51.43 ± 2.26cd	55.36 ± 1.70d
Mineral fertilizer		8.40 ± 1.88a	10.58 ± 0.99bc	122.91 ± 3.28h	132.59 ± 4.44i
Fresh compost tea		12.43 ± 0.29de	11.63 ± 0.63cde	93.65 ± 5.55f	105.41 ± 5.48g
Dead compost tea		12.56 ± 0.65de	11.30 ± 0.40cd	91.85 ± 6.63ef	85.98 ± 4.94e
Statistical significance	Fertilizer	*		*	
	Mycorrhiza	ns		*	
	Interaction	*		*	

There was no significant difference between fresh and dead compost tea in the effect on total P content of non-mycorrhizal and mycorrhizal plants. Total plant P content was also not strongly or consistently affected by AM fungi (Tabs. 5.4.A and 5.4.B).

Total N content of non-mycorrhizal and mycorrhizal plants grown in soil substrate was higher than that of plants grown in peat substrate when compost was amended (Tab. 5.4.A).There was no significant difference between compost distributed homogeneously, in pellet form or in a layer in the effect on plant total N content of non-mycorrhizal plants grown in soil substrate. In contrast, non-mycorrhizal plants grown in peat substrate had a higher plant total N content when this substrate was fertilized with compost distributed homogeneously or in a layer compared to the treatment with compost distributed in pellet form.

Mycorrhizal plants grown in soil substrate had a higher plant total N content when this substrate was fertilized with compost distributed in pellet form compared to treatments where compost was distributed homogeneously or in a layer. In contrast, mycorrhizal plants grown in peat substrate had higher plant total N content when this substrate was fertilized with compost distributed homogeneously or in a layer rather than in pellet form.

Non-mycorrhizal and mycorrhizal plants grown in peat substrate had higher total N contents when this substrate was fertilized with mineral fertilizer or compost tea rather than when it was fertilized with compost (Tab. 5.4.B). Total N content of non-mycorrhizal and

mycorrhizal plants was highest when the peat substrate was fertilized with mineral fertilizer. There was no significant difference between fresh and dead compost tea in their effect on plant total N content of non-mycorrhizal plants grown in peat substrate. However, plant total N content of mycorrhizal plants grown in peat substrate was higher when this substrate was fertilized with fresh compost tea than when it was fertilized with dead compost tea. Inoculation with AM fungi had an increasing effect on plant total N content when the plants were grown in peat substrate fertilized with mineral fertilizer or fresh compost tea.

5.4.5 PHOSPHORUS AND NITROGEN CONCENTRATIONS IN THE SHOOT

Phosphorus concentration in the shoot of non-mycorrhizal and mycorrhizal plants grown in peat substrate was higher than in shoots of plants grown in soil substrate (Tab. 5.5.A). Shoot P concentration of non-mycorrhizal plants grown in soil substrate was higher when this substrate was fertilized with compost distributed in a layer than when it was fertilized with compost distributed homogeneously or in a pellet form. Shoot P concentration of non-mycorrhizal plants grown in peat substrate was lower when this substrate was fertilized with compost homogeneously distributed than when it was fertilized with compost distributed in pellet form or in a layer. In contrast, there was no significant difference between compost distributed homogeneously, in pellet form and in a layer in shoot P concentration of mycorrhizal plants grown in either soil or peat substrate.

Table 5.5.A: Shoot P and N concentration of plants grown in either soil or peat substrate fertilized with compost.

Fertilizer	Growth substrate	Shoot P concentration		Shoot N Concentration	
		-M	+M	-M	+M
Compost distributed homogeneously	Soil	1.53 ± 0.05a	1.50 ± 0.00a	9.42 ± 0.51abc	10.63 ± 0.78bc●
	Peat	1.90 ± 0.24b	1.95 ± 0.17c	8.20 ± 0.94a	7.93 ± 0.67a
Compost distributed in pellet form	Soil	1.40 ± 0.14a	1.53 ± 0.13a	10.20 ± 1.03bc	12.88 ± 3.33c
	Peat	2.23 ± 0.06c	1.83 ± 0.15bc●	9.47 ± 0.91abc	7.88 ± 0.19a●
Compost distributed in a layer	Soil	1.85 ± 0.17b	1.68 ± 0.13ab	11.33 ± 1.91c	9.75 ± 0.51ab
	Peat	2.33 ± 0.29c	2.00 ± 0.24c●	9.23 ± 0.67ab	9.20 ± 1.15ab
Statistical significance	Growth substrate	*	*	*	*
	Fertilizer	*	ns	ns	ns
	Interaction	ns	ns	ns	*

Values are means and SD. Mean values followed by the same letter in the same column are not significantly ($P<0.05$) different. The mean value obtained for a mycorrhizal treatment (+M) followed by a black dot is significantly ($P<0.05$) different from the mean value obtained for the corresponding non-mycorrhizal treatment (-M).

Table 5.5.B: Shoot P and N concentration of plants grown in peat substrate fertilized with compost, mineral fertilizer, or compost tea. For explanation, see Table 5.5.A.

Fertilizer		Shoot P Concentration		Shoot N Concentration	
		-M	+M	-M	+M
Compost distributed homogeneously		1.90 ± 0.24b	1.95 ± 0.17b	8.20 ± 0.94ab	7.93 ± 0.67a
Compost distributed in pellet form		2.23 ± 0.06cd	1.83 ± 0.15b	9.47 ± 0.91abc	7.88 ± 0.19a
Compost distributed in a layer		2.33 ± 0.29d	2.00 ± 0.24bc	9.23 ± 0.67abc	9.20 ± 1.15abc
Mineral fertilizer		1.88 ± 0.15b	1.45 ± 0.17a	28.58 ± 6.44f	18.93 ± 0.77e
Fresh compost tea		1.50 ± 0.00a	1.50 ± 0.00a	11.53 ± 0.75bcd	14.08 ± 0.28d
Dead compost tea		1.55 ± 0.10a	1.50 ± 0.00a	11.73 ± 1.27bcd	11.78 ± 0.75cd
Statistical significance	Fertilizer	*		*	
	Mycorrhiza	*		*	
	Interaction	*		*	

Shoot P concentration of non-mycorrhizal and mycorrhizal plants grown in peat substrate was higher when this substrate was fertilized with compost than when it was fertilized with mineral fertilizer or compost tea (Tab. 5.5.B). There was no significant difference between fresh and dead compost tea in their effect on shoot P concentration of non-mycorrhizal and mycorrhizal plants grown in peat substrate. Plants inoculated with AM fungi had a lower shoot P concentration than uninoculated plants when they were grown in peat substrate fertilized with either compost distributed in pellet and in a layer, or with mineral fertilizer.

Nitrogen concentration in the shoot of non-mycorrhizal and mycorrhizal plants grown in soil substrate was higher than in shoots of plants grown in peat substrate when compost was amended (Tab. 5.5.A). There was no significant difference between compost distributed homogeneously, in pellet form and in a layer in shoot N concentration of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate.

Shoot N concentration of non-mycorrhizal and mycorrhizal plants grown in peat substrate was higher when this substrate was fertilized with mineral fertilizer or compost tea than when it was fertilized with compost (Tab. 5.5.B). Shoot N concentration of non-mycorrhizal and mycorrhizal plants grown in peat substrate was highest when this substrate was fertilized with mineral fertilizer. There was no significant difference between fresh and dead compost tea in their effect on shoot N concentration of non-mycorrhizal and mycorrhizal

plants grown in peat substrate. Plants inoculated with AM fungi had lower shoot N concentration than uninoculated plants particularly when they were grown in peat substrate fertilized with mineral fertilizer.

5.4.6. PHOSPHORUS AND NITROGEN CONCENTRATIONS IN THE ROOTS

Phosphorus concentrations in the roots of non-mycorrhizal plants grown in peat substrate tended to be higher than those of plants grown in soil substrate when compost was amended (Tab. 5.6.A). However there was no significant difference between soil and peat substrate in the effect on root P concentration of mycorrhizal plants when compost was amended. Root P concentration of non-mycorrhizal plants grown in soil substrate was higher when this substrate was fertilized with compost distributed in a layer than it was fertilized with compost distributed homogeneously or in pellet form. There was no significant difference between compost distributed homogeneously, in pellet form and in a layer in the effect on root P concentration of non-mycorrhizal plants grown in peat substrate and of mycorrhizal plants grown in either soil or peat substrate.

Root P concentration of non-mycorrhizal plants grown in peat substrate tended to be higher when this substrate was fertilized with compost than when it was fertilized with mineral fertilizer or compost tea (Tab. 5.6.B). There was no significant difference between mineral fertilizer and compost teas on root P concentration of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate. Plants inoculated with AM fungi often had lower root P concentration than uninoculated plants, particularly when they were grown in peat substrate fertilized with compost distributed in pellet form or grown in soil substrate fertilized with compost distributed in a layer.

Table 5.6.A: Root P and N concentration of plants grown in either soil or peat substrate fertilized with compost.

Fertilizer	Growth substrate	Root P concentration		Root N Concentration	
		-M	+M	-M	+M
Compost distributed homogeneously	Soil	1.58 ± 0.15ab	1.65 ± 0.17ab	7.90 ± 0.43a	9.37 ± 0.76c●
	Peat	1.75 ± 0.25b	1.70 ± 0.00ab	6.88 ± 0.75a	6.66 ± 0.24a
Compost distributed in pellet form	Soil	1.25 ± 0.17a	1.60 ± 0.34ab	7.12 ± 0.87a	8.12 ± 1.16b
	Peat	1.73 ± 0.40b	1.35 ± 0.17a	6.87 ± 1.14a	6.57 ± 0.28a
Compost distributed in a layer	Soil	1.90 ± 0.24b	1.43 ± 0.15ab●	7.83 ± 1.32a	7.98 ± 1.12b
	Peat	1.97 ± 0.25b	1.75 ± 0.29b	7.07 ± 0.36a	7.11 ± 0.56ab
Statistical significance	Growth substrate	*	ns	ns	*
	Fertilizer	*	ns	ns	ns
	Interaction	ns	ns	ns	ns

Values are means and SD. Mean values followed by the same letter in the same column are not significantly (P<0.05) different. The mean value obtained for a mycorrhizal treatment (+M) followed by a black dot is significantly (P<0.05) different from the mean value obtained for the corresponding non-mycorrhizal treatment (-M).

Table 5.6.B: Root P and N concentration of plants grown in peat substrate fertilized with compost, mineral fertilizer, or compost tea.

Fertilizer		Root P Concentration		Root N Concentration	
		-M	+M	-M	+M
Compost homogeneously distributed		1.75 ± 0.25cd	1.70 ± 0.00bcd	6.88 ± 0.75ab	6.66 ± 0.24a
Compost distributed in pellet form		1.73 ± 0.40cd	1.35 ± 0.17ab	6.87 ± 1.14ab	6.57 ± 0.28a
Compost distributed in a layer		1.97 ± 0.25d	1.75 ± 0.29cd	7.07 ± 0.36ab	7.11 ± 0.56ab
Mineral fertilizer		1.43 ± 0.02abc	1.18 ± 0.13a	14.75 ± 3.69f	11.93 ± 0.26d
Fresh compost tea		1.27 ± 0.12a	1.28 ± 0.15a	8.62 ± 0.71bc	9.75 ± 0.18c
Dead compost tea		1.48 ± 0.38abc	1.40 ± 0.14abc	9.36 ± 1.44c	9.37 ± 0.60c
Statistical significance	Fertilizer	*		*	
	Mycorrhiza	*		ns	
	Interaction	ns		ns	

There was no significant difference between soil and peat substrate in their effect on root N concentration of non-mycorrhizal plants when compost was amended (Tab. 5.6.A). There was also no significant difference between compost distributed homogeneously, in pellet form and in a layer in their effect on root N concentration of non-mycorrhizal and mycorrhizal plants grown in either soil or peat substrate. However, N concentration in roots of mycorrhizal plants grown in soil substrate was higher than root N concentration in plants grown in peat substrate.

Root N concentration of non-mycorrhizal and mycorrhizal plants grown in peat substrate was higher when this substrate was fertilized with mineral fertilizer or compost tea compared to the treatments with compost supply (Tab. 5.6.B). Root N concentration of non-mycorrhizal and mycorrhizal plants grown in peat substrate was highest when this substrate was fertilized with mineral fertilizer. There was no significant difference between fresh and dead compost tea in their effect on root N concentration of non-mycorrhizal and mycorrhizal plants grown in peat substrate. Inoculation with AM fungi had no significant effect on root N concentration of plants grown in peat substrate.

5.5 DISCUSSION

In this study, the extent of AM colonization with *Glomus mosseae* was relatively low in both soil and peat substrate when compared to other experiments described in this thesis. Colonization was particularly low when substrates were fertilized with compost or compost tea. The negative effect of compost and compost tea on AM root colonization might be caused by a high P availability in the growth substrates after addition of these materials (Garcia et al., 2007) or by increased microorganism activity in the growth substrate. Other microorganisms may compete with or even parasitize mycorrhizal fungi (Assaf et al., 2009). The present data also shows that the rate of AM colonization in plants grown in soil substrate was lower when this substrate was fertilized with fresh compost tea than when this substrate was fertilized with dead compost tea. This supports the assumption that other microorganisms may have interfered with mycorrhizal colonization. The microbial diversity in the growth substrate was not evaluated, but fresh compost tea may have contained microbes which suppressed the mycorrhizal association.

The extent of AM colonization tended also to be lower in peat substrate than in soil substrate. Vestberg and Kukkonen (2008) reported similarly, that AM colonization was inhibited in peat substrate.The inhibitory impact of the peat substrate on colonization may be due to the high organic matter content, due to high soluble P and/or ammonium, and/or due to the acidity of the peat (Linderman and Davis, 2003). Rhizosphere conditions may be better in peat substrate than in soil substrate, so that plants in peat substrate depend less on the AM symbiosis for growth and nutrient uptake (Corkidi et al., 2004). However, Linderman and Davis (2003) reported also that an increased or decreased AM colonization by peat was dependent on the type of mycorrhizal fungus used.

Very low AM colonization will usually result in very low P uptake via the AM pathway (Smith et al., 2011). In the present study, application of AM fungi did not enhance plant nutrient uptake and plant growth when plants were fertilized with compost or compost tea in both substrates, and this is probably due to the very low AM colonization of these plants. In fact, in the present experiment in some cases inoculation with AM fungi had negative effects on plant growth. Smith et al. (2011) suggested that a negative effect of AM fungi on plant growth is usually due either to the fact that plant growth is C limited (this may be the case in the present experiment for plants grown in soil substrate fertilized with dead compost tea) or to the fact that high P concentrations in the growth substrate alter the characteristic of root colonization, particularly reducing arbuscule development (this may be the case in the present experiment for plants grown in soil substrate fertilized with compost distributed homogeneously). The development of AM fungi in this study was measured by the extent of AM root colonization only, while colonization intensities (number of arbuscules and vesicles per colonized intersection) and hyphae lengths were not recorded. It must also be kept in mind that AM root colonization rates depend not only on fungal activity, but also on root growth rate. Low colonization rates may in part be also due to fast root growth rates, for example in peat substrates with little physical resistance to root growth.

Inoculation with AM fungi enhanced nutrient uptake (P and N) and hence growth of marigold plants when the plants were grown in peat substrate fertilized with mineral fertilizer. In corresponding plants grown in soil substrate P uptake could not be measured due to low biomass. On peat substrate, even though non-mycorrhizal plants had higher shoot P and N concentrations than mycorrhizal plants, mycorrhizal plants had a higher plant total P and N content than non-mycorrhizal plants. This phenomenon may be caused by the fact that dry matter accumulation in mycorrhizal plants was increased more rapidly than the rate of nutrient accumulation, resulting in lower nutrient concentrations in mycorrhizal plants (Jarrell and Beverly, 1981).

Mycorrhizal plants grown in soil substrate fertilized with mineral fertilizer had the highest extent of AM root colonization, but this colonization did not make a positive contribution to plant growth. Probably, the growth of plants in soil substrate was restricted by other factors than P, and furthermore the allocation of photosynthates to the AM fungi might have been limited. Limited allocation of photosynthates to AM fungi impacts the symbiosis (Bücking and Shachar-Hill, 2005). Inoculation with AM fungi in the present study also enhanced the number of flowers and open flowers of plants grown in peat substrate fertilized

with mineral fertilizer (Tab. 5.2). This effect of mycorrhizal colonization on flowering may be linked to faster plant development and better nutrient status in mycorrhizal plants.

The number of flowers is often highly correlated with the size of plants and the biomass (Petit, 2001). Larger plants produce more flowers, and flower earlier than small plants (Mantovani and Iglesias, 2009). In the present experiment, the combinations of growth substrate and fertilizer which supported plant growth best also supported flower formation best. Marigold plants grown in soil substrate had higher plant biomass and hence higher numbers of flowers and numbers of open flowers when this substrate was fertilized with compost than when this substrate was fertilized with mineral fertilizer or compost tea. Marigold plants grown in peat substrate had higher plant DW and hence higher numbers of flowers and open flowers than those grown in soil substrate when this substrate was fertilized with mineral fertilizer or compost tea.

Application of compost to soil substrate resulted in a higher DW of non-mycorrhizal and mycorrhizal plants compared to application of mineral fertilizer or compost teas (fresh and dead compost tea). The increased vegetative growth of marigold plants with application of compost to soil substrate may be due the role of compost in improving soil physical properties. Compost addition can decrease soil bulk density and increase water holding capacity (Caravaca et al., 2002), due to the high content of organic matter in compost (Rivero et al., 2004). Decreasing soil bulk density causes decreasing mechanical resistance of soil which is of high benefit for root growth (Liu and Shan, 2003). This conclusion is also supported by the higher root DW of non-mycorrhizal and mycorrhizal plants grown in soil substrate when soil substrate was fertilized with compost rather than with mineral fertilizer or compost teas. It is not impossible though that the compost used in the present study contained such a high level of nutrients that this in addition supported plant growth.

Many studies reported that plants supplied with nutrients heterogeneously distributed in soil have higher plant biomass production than plants supplied with nutrient homogeneously distributed (Kume et al., 2006; Roiloa and Retuerto, 2006; see also the other chapters of this thesis). However, in the present experiment there was no significant difference between compost distributed homogeneously, in pellet form and in a layer in their effect on total DW and shoot DW of non-mycorrhizal plants grown particularly in soil substrate. Root growth within resource-rich patches was not measured, but total root DW per plant was not affected by compost distribution. This indicates that marigold may have a high ability for nutrient translocation within the plant and nutrient integration for shoot growth. Ma and Rengel (2008) suggested that plant nutrient uptake was controlled by plant nutrient status.

Shoot P or N concentrations of marigold plants were indicative of deficiency when compared to standard values (Munson, 1998). Root P and N concentrations were also low. Plants deficient in a certain nutrient may take up this nutrient from soil when available to them, irrespective of homogeneous and heterogeneous distribution of this nutrient in the soil. There was no difference in the present experiment in biomass production between soil substrates with homogeneous and heterogeneous compost distribution. This may be due to the fact that efficiency of root foraging in the nutrient rich patches was reduced, because of nutrient depletion in these patches (Kembel and Cahill, 2005) at the end of the experiment.

There was no significant difference between application of mineral fertilizer and compost teas (fresh and dead compost tea) in the effect on DW of non-mycorrhizal and mycorrhizal plants grown in soil substrate. Compost teas have no solid organic matter particles. Thus, applications of compost teas clearly have less effects on soil physical properties than compost applications.

The type of growth substrate in the present study clearly affected nutrient uptake and hence plant growth. Non-mycorrhizal and mycorrhizal plants grown in peat substrate had higher plant DW than corresponding plants grown in soil substrate, particularly when both substrates were fertilized with mineral fertilizer or compost teas. As plant growth substrate, peat has lower bulk density, provides better aeration, and has a higher water holding capacity than mineral soil. Plant growth may benefit from peat rhizosphere conditions more than from soil rhizosphere conditions (Corkidi et al., 2004). In addition, peat has a higher cation exchange capacity than soil substrate, so that peat has a good capacity to store cationic nutrients for the plants. Phosphates in compost are often easily available to plants (Maher et al., 2008). In the present study, plants grown in peat substrate had lower shoot/root ratios than plants grown in soil substrate (Fig. 5.1.B), due to the intense root growth on peat.

In contrast with the beneficial effects of applications of compost to soil substrate, applications of compost to peat substrate resulted in lower plant nutrient uptake (particularly N uptake) and hence lower plant growth than applications of compost teas. Beinga liquid, compost tea has very little effect on the physical properties of peat substrates, so that the porosity of the peat substrate is maintained (Scheuerell, 2004). In contrast, solid compost has a relative fine texture and high density compared with peat. The mixture between compost and peat may in some cases have a negative effect on plant growth because this mixture has a lower porosity than peat only (Veijalainen et al., 2008). In addition, in the present study non-mycorrhizal and mycorrhizal plants grown in peat substrate had lower plant nutrient content and DW when this substrate was fertilized with compost distributed in pellet form rather than

with compost distributed homogeneously or in a layer. Compost pellets may be very compact, so that accessibility of nutrients in the short- and medium-term is less than in non-pelleted compost. Pelleted compost can thus be recommended only for long-term fertilization.

The effects of applications of fresh and dead compost tea were not significantly different in terms of plant nutrient content and concentration and hence DW of non-mycorrhizal or mycorrhizal plants grown in either soil or peat substrate. The effect of applications of bacterial inoculants from fresh compost tea to stimulate plant growth in another study was affected by the nutrient condition of the growth substrate (Egamberdiyeva, 2007). In that case, bacterial inoculation had a much better effect to stimulate plant growth in nutrient deficient soil than in nutrient rich soil. In the present study, there was likely no or only little nutrient deficiency in growth substrates fertilized with compost teas, because non-mycorrhizal plants grown in peat substrates had higher P contents and hence DW when this substrate was fertilized with compost tea than when it was fertilized with mineral fertilizer.

The findings of the present study clearly indicate that conventional mineral fertilization in the cultivation of marigold could be replaced by application of compost, so that further environmental pollution by mineral fertilizers could be avoided. However, the type of compost must be considered in regard of the type of growth substrate when compost is to be applied. Application of compost tea gives more benefit to plant growth and flowering when it is applied in peat substrate rather than in soil substrate. In contrast, application of compost gives more benefit to plant growth and flowering when it is applied in soil substrate rather than in peat substrate. Further tests are necessary to determine AM inoculants which perform well in different substrates, so that synergistic effects occur between AM fungus, type of fertilizer and growth substrate. Then, the benefit of using biological and organic fertilizers at the same time can be fully realized.

6. GENERAL DISCUSSION

6.1 EFFECT OF SOIL CONDITIONS ON THE EXTENT OF ARBUSCULAR MYCORRHIZAL ROOT COLONIZATION AND ON THE DEVELOPMENT OF EXTRARADICAL HYPHAE

The importance of mycorrhizal fungi in the mineral nutrition of plants depends on the ability of the fungi to exploit sources of non-mobile nutrients in the soil (Becerra et al., 2007). Therefore, the symbiotic efficiency depends not only on plant and fungal genotype, but also on soil factors such as forms and amounts of non-mobile nutrients in the soil, and on the interaction between all these factors. For example, soil fertility is also an important factor in the control of mycorrhizal colonization (Correnho et al., 2007).

At high soil P concentration, the extent of AM root colonization is usually inhibited, because carbon allocation to the AM fungi from the host plant is reduced (Gosling et al., 2013). In agreement, in the present study the extent of AM root colonization was higher when plants were supplied with low mineral P than with high mineral P (Chapter 2; Fig. 2.6).

It has to be kept in mind though, that plant nutrient distribution in soil at natural sites or in practical agriculture is usually not homogeneous.

In one of the experiments described in this thesis, root AM fungal colonization by*G. intraradices* was not affected by differences in P supply to the two halves of the root systemin a split-root experiment (Chapter 3; Tab. 3.2.A-D). This indicates that the P status of the host plant (shoot) rather than local soil P supply determined colonization rates. In that experiment, shoot P concentration of plants inoculated with *G. intraradices* was not affected by the distribution of P supply in the root compartment (Tab. 3.3.A). Notably, even root P concentrations were not affected by variations of the P distribution in the soil (Tabs. 3.3.B and C). Jarosch et al. (2008) also suggested that the extent of root colonization dependsprimarily on the P status of the host plant. This is not surprising as the plant P status regulates the carbohydrate allocation to the root (Hammond and White, 2008).

In many cases, AM colonization is highest at moderate soil P concentrations (Olsson et al., 2006). The highest level of P supply in the root compartments in the experiment reported in Chapter 3 was 85 mg P per kg dry soil, and this amount was apparently not high

enough toinduce a local reduction of AM fungal colonization by *G. intraradices*. From the results of this thesis, it is unclear whether extremely high P concentrations in soil patches would result in locally decreased colonization of the root by *G. intraradices*.

In contrast to *G. intraradices*, in the present study AM fungal colonization of *G mosseae* was significantly affected by P supply in the root compartment. In a split-root experiment (Chapter 4), both a higher and a lower level of P supply in aroot compartmentslightly decreased the extent of AM fungal colonization by *G. mosseae* compared to the treatment with equal P supply in both root compartments (Tabs. 4.2.B and C). Decreasing AM fungal colonization in the root compartment that received a higher amount of P might be caused by increasing root growth which will decrease the ratio of colonized to un-colonized roots in that part of the root (Smith et al., 2011). Decreased AM fungal colonization at low local P supply might be caused by competition between the host plant and the AM fungus for the limited amount of P in supply (Peters and Habte, 2001).

In the present study, the activity of the AM fungal mycelium in P uptake from hyphal compartments wasapparently also not specifically affected by different P supply distribution in soil. Thisapplied to both *G. intraradices* and *G. mosseae* (Tabs. 3.2.B and C; 4.2.B and C). Phosphorus uptake by hyphae was not measured directly, but the activity of the AM fungal mycelium in P uptake was estimated from the ratio of coarse (runner hyphae) to thin (absorbing hyphae) hyphae according to Olsson et al. (2006). Previously published evidence also suggests that plant P status controls the rate of AM fungal P uptake (Nagy et al., 2009). It should be noted, however, that in the present study measurements of the ratio of coarse to thin hyphae had in many cases high standard errors, so that the lack of a significant treatment effect is not good evidence of a general lack of influence of local P supply on hyphal uptake activity.

The development of extraradical hyphae of the AM fungus *G. Intraradices* may be more sensitive than the development of intraradical colonization of that fungus to low versus high soil P concentration. Alower P supply increased the weight of mycelium (significantly) and the hyphae length (in tendency) in the hyphal compartment (Tab. 3.2.B). Under decreasedsoil P supply, carbon flow to the fungus can beincreased, and lipid transport from intraradical to extraradical mycelium can also be increased (Olsson et al., 2002). Lipids are important for energy storage and the main component of fungal biomass (Olsson, 2009).

In contrast, in most other measurements in this thesis the length of extraradical hyphae and the weight of the mycelium were not significantly affected by a local increase in P supply. This probably indicated that the growth of AM fungi was not limited by P

deficiency in the part of the soil that received alower amount of P, and that both AM fungi did not specifically forage in the root compartment that received the higher amount of P.

The extent of AM root colonization and the weight of extraradical mycelium from both isolates were also not affected by the N supply ratio (Chapter 3 and 4; Tabs. 3.2.E and F, Tabs. 4.2.E and F). The most important factor influencing mycorrhizal symbiosis is usually soil P availability (Nogueira and Cardoso, 2007). However, when compared to homogeneous soil N distribution in the present study the number of spores per unit mycelium dry weight was decreased in the soil half with higher N supply in one of the split-root experiments (Tabs. 3.2.D-F) while the number of spores per unit mycelium length was decreased in the soil half with lower N supply in another experiment (Tab. 4.2.E). Thus, spore production per unit mycelium was affected by N supply in the present study, but the effect was not consistent. The decreasing number of spores in the soil zone that received the higher amount of N might be caused by increasing N assimilation of the fungus (Wallenda et al., 1996). Conversely, the decreasing number of spores in the soil zone that received a lower amount of N might be caused by lower N uptake for spore production. Nitrogen is the main component of chitin which is a component of spore walls (Bago et al., 2004; Roesti et al., 2005).

In summary, in the split-root system used in experiments described in Chapter 3 and4, the effects of soil P and N distribution on mycorrhizal colonisation and spread were not very distinct and not consistent. It should be kept in mind that variations of nutrient distribution to two halves of a root system (as in experiments described in Chapter 3 and 4) may have different effects from small-sized local soil patches ("hotspots" of nutrients) with high nutrient supply intensity.

In the present study, the extent of root colonization by *G. mosseae* was particularly low when marigold plants were grown in peat (Chapter 5; Tab. 5.3). Although peat has superior physical and hydraulic properties for plant growth (Raviv and Lieth, 2008), high organic matter, high soluble P contents, high ammonium concentrations and the acidity of peat may all be reasons for the inhibition of AM root colonization in peat-grown plants (Linderman and Davis, 2003).

6.2 EFFECT OF FERTILIZER TYPE ON THE EXTENT OF ARBUSCULAR MYCORRHIZAL ROOT COLONIZATION AND ON PLANT GROWTH

Mineral nutrients are required in adequate amounts for optimum plant growth. Plants absorb mineral nutrient particularly in inorganic form. In the present study, mineral nutrient forms, organic material, compost, and aqueous extractsof composts(so called compost tea; fresh and dead) were used as nutrient supply treatments (Chapter 2, 3, 4, and 5).

The ability of the plants to absorb P depends on the concentration of P ions in the soil solution at the root surface and the area of absorbing surface in contact with the solution (Grant et al., 2005). Application of small amounts of mineral P in combination with inoculation withAM fungi in the present study increased plant dry weight and plant P and N uptake in similar magnitude as an application of a high amount of mineral P (Chapter 2). This clearly supports the view that application of AM fungal inoculum may reduce the need for mineral fertilizer use.

In the present study, AM fungi did not in general respond positively to organic matter amendments in soil. The extent of AM root colonization was decreased when mycorrhizal roots encountered plant organic matter in soil (Chapter 2; Fig. 2.6). The decreasein the extent of AM root colonization in that case may be caused by increasing nutrient concentrations in soil during the decomposition process (Correnho et al., 2007). Organic matter amendments mayalso increase microorganism activity in the growth substrate, and these microorganisms may then compete with or even parasitize AM fungi (Assaf et al., 2009). Secondary metabolites produced by microorganisms involved in organic material decomposition may also be harmful to AM fungi (Gryndler et al., 2009). Potentially, AM fungal hyphae can also acquire mineral nutrients from decomposing organic matter and perhaps even accelerate mineralization of organic matter, but these processes have not been well investigated (Neumann and George, 2010).

Compost as well as compost tea application to soil did not stimulate AM root colonization in the present study (Chapter 5; Tab. 5.3). This result is in accordance with Üstüner et al. (2009). They found that root colonization rates were even significantly lower when the growth medium was supplied with 10, 30 or 100% compost. However, in contrast many other studies reported that plants grown in soil supplied with compost had a higher AM root colonization, compared with plants grown in compost-free soil (for example, Valarini et

al., 2009, Tanwar et al., 2013). Ortas et al. (2009) reported that the response of AM fungi to compost differed depending on the compost material and its nutrient content. The extent of AM root colonization was even decreased in soil fertilized by compost in the present study (Tab. 5.3). This might be caused by high P availability in that substrate (Garcia et al., 2007). Üstüner et al. (2009) suggested that selection of compost amendment and a suitable AM fungal isolate is critical to obtain a synergy between compost and AM fungi for optimum plant growth.

In the experiments described in this thesis, plant dry weight and P uptake was increased by application of organic matter or compost to soil. Plants grown in compost-amended soil hadmuch higher plant dry weight and nutrient uptake than plants grown in soil supplied with mineral nutrients or compost tea (Tab. 5.1.A). Soil physical properties such as soil bulk density and water holding capacity can be improved by adding compost to soil. Compost supply often reduces soil bulk density and increases water holding capacity, hence giving a benefit for root growth. The present study clearly supported the well-known positive effects of compost supply, but didnot give any indication that AM fungi may be specifically involved in this beneficial compost effect.

6.3 EFFECT OF THE FUNGAL ISOLATE AND OF BACTERIA ON PLANT GROWTH AND NUTRIENT UPTAKE

Mycorrhizal fungi form a mutually symbiotic relationship with most terrestrial plants. By their extensive hyphal network, AM plants have a higher ability to exploit the soil volume compared to non-mycorrhizal plants. In agreement with this expectation, plants inoculated by AM fungi had higher P and N uptake and hence plant dry weight than un-inoculated plants in the present study (see, for example, Figs. 2.2, 2.7 and 2.8).

The mycorrhizal effect on plant nutrient uptake and growth is, however, quite variable. Two of the factors that contribute to the divergence of AM fungal effects are the plant and fungal genotype (Redon et al., 2009). The AM fungus-plant associations are known to be in general non-host specific. Despite the non-specificity of this association, certain fungus-plant associations are more efficient than others (Twun-Ampofo, 2008). In general terms, the development of AM fungi depends on the soil conditionsbecause AM fungi must adapt to their soil environmentto be successful (Pánková et al., 2011).

In the present study, the contribution of AM fungi of different origin (from long-term

minerally and organically fertilized soil) (Chapter 2) and of different species *(G.intraradices* and *G. mosseae*) (Chapter 4) to growth of sweet potato plants was investigated. The species of AM fungi from long-term minerally and organically fertilized soil were not identified, but it was expected that long-term differences in fertilization would induce a shift of mycorrhizal fungal types. Plants inoculated with AM fungi from either minerally or organically fertilized soil were grown in soil supplied with either mineral or organic fertilizer.In another approach, plants inoculated with either *G. intraradices* or *G. mosseae* were grown in soil supplied with mineral fertilizer. Both *G.intraradices* and *G. mosseae* inocula were obtained from pot cultures of the respective fungal isolate with maize plants on the same soil as used in the experiment.

In the present study, there was no significant difference in the effect of AM fungi from long-term minerally or organically fertilized soil in their contribution to P and N uptake and growth of plants which were grown in soil fertilized with either mineral P or organic material (Chapter 2). This indicated that perhaps AM fungal diversityin the field was not affected by differences in long-term fertilization. This, however, is unlikely in view of much published evidence from other research groups. More likely, the different AM fungal types were able to quickly adapt to the current P supply condition in the experiment. The extent of AM root colonization was also not significantly different between AM fungi from minerally and organically-fertilized soil. The root colonization of plants by AM fungal inoculum is not always dependent of the origin of the AM fungi (Pánková et al., 2011). In conclusion, this study did not result in any evidence that AM fungi isolated from soil with a long history of organic matter application have and conserve superior properties in the exploitation of organic nutrient sources for the plant.

Plants inoculated with *G. intraradices*showedan increased plant dry weight and P content compared with plants inoculated with *G. mosseae* (Chapter 4; Tabs. 4.1.A and 4.3.A). The higher effectiveness of *G.intraradices* compared with *G. mosseae* to increase nutrient uptake and plant growth in the present study might be related to differences in the extent of AM colonization and in the development pattern of extraradical mycelium. *G. intraradices*causeda significantly higher AM root colonization compared with *G. mosseae* (Chapter 4; Tabs. 4.2.B, C, E and F). The low root AM colonization rates in marigold plants colonized by *G. Mosseae* (Tab. 5.3) did not have a positive effect on plant growth and P uptake. Very low AM colonization usually results in very low P uptake via AM pathways (Smith et al., 2011). Liu et al. (2008) also reported that under low soil P conditionsa higher extent of AM root colonization resulted in a higher contribution to P uptake in soybean

inoculated with *G. mosseae*.

The development pattern of the extraradial mycelium was different between *G. intaradices* and *G. mosseae*. The thin (absorbing) hyphae were more numerous in *G. intraradices* than *in G. mosseae.* On the contrary, the coarse (runner) hyphae were more numerous in *G. mosseae* than in *G. intraradices* (Tabs. 4.2.B, C, E and F). The physiology of hyphae and the capacity to absorb and transport nutrients to the host is probably related to the diameter of the hyphae. A smaller diameter allows hyphae better to proliferate and to absorb nutrients from soil (Chaudhry et al., 2012).

Arbuscular mycorrhizal fungi continuously interact with a wide range of soil microorganisms, including soil bacteria (Miransari, 2011). Plantavailability of soil P can be increased due to inorganic P release from decomposed organic matter as a result of activity of soil microorganisms (Prescot, 2005). This inorganic P released from decomposed organic matter can then be taken up by AM fungi and hence increase plant growth. However, application of bacteria in the present study did not increase plant growth, even when plants were supplied with organic matter (Fig. 2.2). This contradicts findings in many other studies on beneficial effects of bacterial inoculation. Of course, in the present study bacteria were also present in experimental pots that were not inoculated with bacteria. Bacteria can easily spread with wind and water, and the present experiments were not carried out under sterile conditions. Practical agriculture also uses non-sterile substrates, and the present results do not support the assumption that bacterial inoculation will be helpful under these conditions.

6.4 EFFECT OF SOIL NUTRIENT DISTRIBUTION ON PLANT GROWTH AND NUTRIENT UPTAKE

As a main focus of this study, plants inoculated or not with AM fungi were grown with supply of organic material (Chapter 2), mineral P or N (Chapter 3 and 4) or compost (Chapter 5) distributed either homogeneously or heterogeneously in the growth substrate. Within each experimental approach, the same quantity of nutrients was supplied but spatial distribution in soil was different, to allow investigation of the effects of soil nutrient distribution on plant growth and nutrient uptake.

Theresults showed that both mycorrhizal and non-mycorrhizal plants supplied with organic material heterogeneously distributedcan havein some situations a higher plant dry weight and nutrient uptake compared with plants supplied with organic

materialhomogeneously distributed (Fig. 2.2). Plants supplied with organic material distributed heterogeneously had an increased proportion of total root dry weight in the patches (Fig. 2.5). This indicates that the experimental plants responded to nutrient heterogeneity by root proliferation in the nutrient patch. By this root proliferation in the patch, plants optimize the uptake of nutrientsfrom within that patch.

This root proliferation in the patch was not clearly different between mycorrhizal and non-mycorrhizal plants (Fig. 2.5 and Tab. 2.2.B). Mycorrhizal plants had a higher plant dry weight and nutrient uptake than non-mycorrhizal plants, but AM fungi in this study did not specifically help the plant to respond to local organic materialsupply (Chapter 2). A similar observation was made in plants supplied with a different distribution in soil of P or N. The AM fungi also in this case did not specifically forage nutrients in soil zones that received a spatiallyhigher amount of either P or N (Chapter 3 and 4). Neumann and George (2010) suggested that AM fungi may assist host plantsin the exploitationof patchy soil either by increasing exploitation resources in the patch or by increasing uptake capacity outside the patch.

Plant response to heterogeneous nutrient distribution in soil was not consistent across all experiments in this thesis. There was not always a significant effect of homogeneous versus heterogeneous nutrient distribution in plant P and N uptake and plant dry weight in the experiments reported in Chapter 3, 4, and 5 of this thesis. In split-root systems, root growth responded positively to local higher N supply (Tabs. 3.1.D and 4.1.D), while localized P supply affected root distribution significantly in one experiment (Tab. 4.1.A) but not in another study using *G. intraradices* only (Tab. 3.1.A). Total root dry weight not significantly affected by mineral P or N distribution in soilin the split-root system (Tabs. 3.1.A and D, 4.1.A and D). Compost distributed in a layer in a soil substrate caused increased total root dry weight of mycorrhizal plants compared to compost distributed homogenously in soil (Tab.5.1.B).

These variations in response to local nutrient supply may be related as least partly to the different size of the nutrient rich soil patch in the present study. The volume of the patches in Chapter 2 and in Chapter 3 and 4 were approximately 5% and 50% of the total volume of the bulk soil, respectively. Hutching and Wijesinghe (1997) reported that plants had higher biomass production under heterogeneous nutrient distribution if the nutrients were concentrated in small patches. In such small patches, relatively less soil adsorption of nutrients may take place than in larger patches where the added nutrients are more diluted in soil. In this case, plants benefit more from the exploitation of small, intense nutrient patches.

In contrast, Kume et al. (2006) reported that also larger P patches can cause enhanced plant dry weightdue to increasing root length in the patches. In general, plants may tend to have the same growth rate on soils with nutrients distributed homogeneously or heterogeneously, because in the long term plant (shoot) nutrient status regulates plant nutrient uptake (Ma and Rengel, 2008). In that situation, plant nutrient uptake is more regulated by shoot demand rather than by variations in the soil distribution of nutrients.

7. SUMMARY

Under nutrient limiting conditions, the organ that mostly affects the plants ability to grow and to survive is the root system. The size of the total absorptive area of the root system determines the plant capacity for uptake of nutrients. In this context it is important to consider thatroots of most terrestrial plant species are colonized by AM fungi. This colonization assists plants in nutrient uptake from soil. But nutrient distribution in the soil is never uniform, although almost all pot-based experiments in plant nutrition are carried out on homogeneous substrates. One of the factors that are causing nutrient heterogeneity in soil is the decomposition of organic matter, assisted by soil bacteria. Therefore, it is very important to understand the role of AM fungi and their effect on the modification of the plant root system, perhaps resulting in an increase in nutrient uptake in situations of heterogeneous nutrient distribution in soil.

To investigate the role of AM fungi in nutrient uptake in situations of heterogeneous nutrient distribution in soil, plants inoculated or not inoculated with AM fungi were supplied with organic material (Chapter 2), mineral P or N (Chapter 3 and 4) or compost (Chapter 5) distributed either homogeneously or heterogeneously in the substrate. The quantity of nutrientssupplied was always similar between treatments with homogeneous and with heterogeneous nutrient distribution. Arbuscular mycorrhizal fungi from long-term minerally and organically fertilized soil (Chapter 2), or isolates of *G. intraradices* (Chapter 3 and Chapter 4) and *G. mosseae* (Chapter 4 and 5) were used as AM fungal inoculants. In one experiment, bacteria from a long-term organically fertilized soil were applied as bacterial treatment (Chapter 2).

In most experiments described in this thesis, AM fungi increased plant P uptake and plant growth, and this effect was irrespective of nutrient distribution in the substrate (Chapter 2, 3 and 4). An exception was the experiment described in Chapter 5 where the extent of root colonization by AM fungi was low and AM fungi did not contribute to plant growth in most treatments. This was probably due to the application of compost or compost tea in that experiment, and/or an incompatibility of the AM fungus used in that experiment with compost as a nutrient source.

The extent of AM root colonization was in some cases reduced when roots encountered organic material in soil (Chapter 2), but was not affected by differencesin P or N supply to two parts of the root system (Chapter 3 and 4). The extent of AM root colonization was mainly affected by the plant P status. The P concentration in the shoot was not affected

by the local distribution of P or N supply over the two parts of the root system in split-root experiments (Chapter 3 and 4). This thesis did not collect evidence that nutrient rich patches or organic material arespecifically exploited by AM fungi. AM fungi might, however, assist plant growth by increasing uptake capacity from inside and outside the nutrient rich patches.

In the present study, different long-term application of fertilizer (mineral and organic) in the field had no effect on the ability of AM fungi originating from these fields to colonize plants and to increase plant growth (Chapter 2). However, in another experimentof this study different species of AM fungi differed in their contribution to plant nutrient uptake and growth. An isolate of *Glomus intraradices* had a higher capacity to increase plant P uptake and hence plant dry weight compared to an isolate of *G.mosseae.* This was related to the extent of AM root colonization and the development pattern of the extraradical mycelium. *Glomus intraradices* had a higher extent of AM root colonization and produced more thin (absorbing) hyphae than *G. mosseae* (Chapter 4).

Plant P and N uptake and plant growth were not affected by bacteria inoculation. This was true even when soil was supplied with organic matter (Chapter 2). The experimental design did not allow a sterile environment, and in open environments a high number of bacteria will be present in the substrate even without inoculation.

The size of the nutrient rich patch and the plant nutrient status might both affect growth and nutrient uptake of plants growing on soils with heterogeneous nutrient distribution. In the present study, plants grown in substrate with heterogeneous nutrient distribution,(i.e. small sized organic matter patches were inserted) in some cases showed higher P and N uptake ratesas well as higherplant dry weight compared to plants grown in soils with homogeneous nutrient distribution.(Chapter 2).In contrast, there was mostly no significant difference in P and N uptake and growth of plants when plants were supplied with different P or N distribution ratios over two halves of a root system, with a much larger nutrient rich zone (50% of the total soil volume; Chapter 3 and 4). Plant growth and nutrient uptake were also notaffected by different compost distributions in soil (Chapter 5).A direct comparison between experiments in this study has to be done with some care though, because the host plant species in the experiment described in Chapter 5 (marigold) was different from the host plant species used in the other experiments (sweet potato).

Application of compost resulted in a higher plant dry weight compared with an application of either mineral fertilizer or compost tea when plants were grown in soil substrate. In contrast, application of compost resulted in lower plant dry weightcompared to application of compost tea when plants were grown in peat substrate. There was no

significant difference in plant growth between application of mineral fertilizer and compost tea when plants were grown in soil substrate (Chapter 5). Application of compost also reduced the extent of AM root colonization. Compost application was beneficial to plant growth due to both chemical and physical properties, but this study did not show that compost specifically supports mycorrhizal functions.

I conclude that plants can benefit from heterogeneous nutrient distribution in soil if plant roots can exploit the nutrient sources in the nutrient-rich patches. The size of the patches and the type of nutrient source in the patch must be considered when predicting benefits to plant growth. Application of AM fungi increased P uptake and hence plant growth in situations of both heterogeneously and homogeneously distributed soil nutrients. Arbuscular mycorrhizal fungi did not show a specific foraging activity in the nutrient-richsoil patches. Application of AM fungi and/or of organic material (or compost) both can help to reduce mineral fertilizer use. However, type of organic material (or compost) and AM fungal species must be carefully selected and substrate conditions must be considered before an application of organic material and/or AM fungi will result in optimum plant growth.

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Erklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung derangegebenen Literatur und Hilfsmittel angefertigt habe

Wahyu Harso, Palu